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THE INDUCTION OF PARTHENOCARPY IN PETUNIA[1]

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Numerous attempts to induce haploidy in plants have been made in the past, and the techniques have varied widely. These have included, among other methods, hybridization, both intergeneric and interspecific (Clausen and Mann, 1924; Gaines and Aase, 1926); cold and heat treatments (Blakeslee et al, 1922; Belling and Blakeslee, 1927; Randolph, 1932); injury to plant parts (Davis, 1931; Ivanov, 1938); irradiation of pollen with x-rays (Katayama, 1934; Ivanov, 1938; Rick, 1943); application of various sorts of pollen (Belling and Blakeslee, 1927; Jörgensen, 1928); and chemical treatments (Gustafson, 1936, 1942; van Overbeek et al, 1941). This report is concerned with three of these methods as they affect fruit development: the application of different pollen types and chemical and x-ray treatments. The plants from seed produced in the x-ray experiments will be dealt with in a later report.

PARTHENOCARPY INDUCED BY VARIOUS POLLENS

The effects of pollen extracts have been of some interest since the work of Fitting (1909) and Laibach (Laibach, 1933; Thimann, 1934). Redinger (1938) reported the production of homozygous diploids in *Petunia* through the application of pollen of closely related solanaceous forms. It was decided for this study to apply some pollen from plants bearing no close relationship to *Petunia* as well as some from closely related genera. Table I gives the results obtained.

Materials and Methods.—*Petunia* flowers were emasculated and pollinated with "foreign" pollen. Except where orchid pollinia were used, contamination was prevented by placing a piece of soda straw closed at one end with Scotch tape over the stigma and style. This could not be done with the pollinia for danger of dislodging them. All pollinia from a single orchid bloom were used in each

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treatment. The ovaries were allowed to remain on the plant until dried. After harvest, the thickness, texture, and shape of the ovary walls and activation of the ovules were examined under the binocular microscope and compared to those of normal fruits. Measurements were made along the long and short axes in millimeters. Controls were not pollinated after emasculation but stigmas and styles were covered. Only those treatments which gave positive results are cited below.

Treatments with orchid pollen.—Since the experiments of Fitting and Laibach, orchid pollen has been credited with containing relatively large amounts of some substance or substances, or the precursors of such substances, which initiates development of the ovary.

TABLE I

Pollen sources	Petunia strains pollinated											
	1A	2	2A	3	4	6	LaPal*	BT	Son	Noc	Total	Results
	Number of times used											
Cattleya Mossiae plus stigmatic substance						1					1	—
Stigmatic substance of *Cattleya Mossiae*					4	1					5	—
Cattleya "Priscilla" plus stigmatic substance				3	4	6					13	1+
Cymbidium sp.				16	11		2				29	4+
Cymbidium plus stigmatic substance				7	7	7					21	6+
Delphinium sp.	1	4		1					1		7	—
Lilium longiflorum	2	4	1	8		1					16	—
Lilium tigrinum	8		9	11	8	8	1	8			53	—
Paeonia sp.		6									6	—
Philadelphus sp.	1		2	4	6	2	2	1		1	19	—
Lycopersicum esculentum	1	5	3		1		5				15	—
Nicotiana affinis	1	10	4	9	19	26	7	18			94	4+;5PF
Nicotiana glutinosa				10							10	—
Nicotiana Tabacum				15							15	1+
Salpiglossis sp.				45							45	2+;1PF
										Total	349	

*Symbols: LaPal = La Paloma.
BT = Better Times.
Son = Sonata.
Noc = Nocturne
PF = Parthenocarpic fruit.
+ = Some activation.
— = No activation.

Results.—No parthenocarpic fruits were produced. Eleven of the treated ovaries showed mild activation, ten in the form of thickness and texture changes in the upper one-third to one-half of the wall. There was no increase in size. Only in one case was there any activation of the ovules. This ovary exhibited a texture and thickening change in the upper half of the walls (pl. 11, fig. 1), while two ovules at the top of the column developed sufficiently to be classified as distorted empty seeds.

In general, the activation of *Petunia* ovaries with orchid pollen appears to be very slight. It seems possible to bring about such slight activation in the walls without affecting the ovules. In the case where the ovules were activated, the orchid pollinia were accompanied by stigmatic substance.

Treatments with pollen of Nicotiana affinis.—Of the four activated ovaries, one showed a partial hardening of the upper one-third of the ovary wall; three showed a hardening in the upper tip of the wall accompanied by a slight activation of a few ovules at the top of the column. The five parthenocarpic fruits were smaller than normal fruits (5 × 3½ mm., 4½ × 4, 4½ × 3, 3½ × 3, 3 × 3). All the walls exhibited the thickness, texture, and shape of normal fruits (pl. 11, fig. 2). They contained hollow seeds and split when ripe. Two of these fruits contained some ovules which had apparently undergone lesser degrees of stimulation and had developed in some cases to flat and distorted integumental structures (pl. 12, fig. 2).

Treatments with Nicotiana Tabacum.—One ovary showed a hardening and thickening in the upper third of the walls; the ovules were unchanged.

Treatments with Salpiglossis pollen.—Three ovaries gave positive results. In two of these the upper one-third of the capsule showed a hardening on the outside; the inner surface was not shiny as in a normal mature fruit. Neither was any larger than an unpollinated ovary allowed to dry on the plant (2½ × 1½ mm., 3 × 1½). The size of the third fruit (4½ × 3½ mm.) indicated greater activity. The upper three-fourths of the wall had hardened and thickened; the inner surface had become somewhat shiny but ovules showed no activation.

PARTHENOCARPY INDUCED WITH 2, 4-D

The effectiveness of 2, 4-D in the production of parthenocarpic fruits has been amply demonstrated (Avery, 1947). Of the chemical substances used in this study in attempts to stimulate development of the egg, 2, 4-D, although giving no results parthenogenetically, did produce some interesting results parthenocarpically. In an initial test, 2, 4-D at 2 p.p. 100 in lanolin was applied to the stigmatic surfaces of 21 emasculated flowers and in all cases gave positive results. For the most part, these fruits were perfectly normal in appearance, splitting at maturity to reveal an abundance of hollow seeds. A few of these seeds, when punctured with a needle, were seen to have a small amount of whitish material inside. The three largest fruits measured 7 mm. along the long axis and 4 mm. along the short; the remainder showed a gradual decrease in size to the smallest which was 3½ × 2½ mm. Only one of these fruits (6 × 4 mm.) did not contain at least a few empty seeds, but contained only ovules which had obviously undergone an activation where development of the integument had fallen short of the hollow-seed stage.

Because of the pronounced effect of 2, 4-D at such a high concentration, it seemed advisable to check it at lower levels and in different media; accordingly, tests were run using the substance in lanolin, water and talc at concentrations of

1 p.p. 100,000, 1 p.p. 10,000, 1 p.p. 1,000, 1 p.p. 100 and 2 p.p. 100 in each medium.

Materials and Methods.—The pure acid was ground and mixed in lanolin or talc to the desired concentration; when water was used as a medium the material was dissolved in a few cc. of acetone and then properly diluted with distilled water. The paste, powder, or liquid was then applied to the stigmatic surfaces of emasculated flowers; contamination by pollen was prevented by the straw method.

TABLE II

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
			2, 4-D in lanolin 1 p.p. 1000	
6	2½ × 1½	—	—	—
6	3 × 2½	Small PF	Some activated	Th, Tex, S
6	3 × 2½	Small PF	1 Hol. S, remainder activated	Th, Tex, S
6	5 × 4	PF	Hol. S	Th, Tex, S
6	4 × 2	Small PF	Activated	Th, Tex, S*
6	3½ × 3	Small PF	Hol. S	Th, Tex, S
6	4½ × 3	Small PF	A few Hol. S (distorted)	Th, Tex, S (upper ¾* of length, papery below)
6	3½ × 2	Small PF	Some activity opposite active part of wall	Th, Tex, S (upper ¾* of length, papery below)
6	3½ × 1½	Small PF	As above	As above*
6	3 × 2	Small PF	As above	As above*
			2, 4-D in lanolin 1 p.p. 100	
6	3½ × 1½	—	—	—
3	4 × 2½	—	—	—
6	3 × 2	—	—	—
6	4 × 1½	—	—	—
6	4 × 3	Small PF	Some activity opposite active part of wall	Th, Tex, S upper ¾*, papery below
6	6 × 4	PF	Some Hol. S, remainder active	Th, Tex, S
6	7 × 5	PF	Abundant Hol. S	Th, Tex, S
6	9 × 5½	PF	Some small round Hol. S, remainder active	Th, Tex, S
6	10 × 5	PF	Abundant Hol. S	Th, Tex, S
6	8 × 5	PF	Abundant Hol. S	Th, Tex, S
6	8 × 4	PF	Abundant Hol. S	Th, Tex, S
			2, 4-D in lanolin 2 p.p. 100	
6	3½ × 1½	—	—	—
6	2½ × 1½	—	—	—
6	3½ × 1½	—	—	—
6	6 × 5	PF	Abundant Hol. S	Th, Tex, S
6	7 × 6	PF	Abundant Hol. S	Th, Tex, S
6	7 × 6	PF	Abundant Hol. S	Th, Tex, S
6	6½ × 6	PF	Activated	Th, Tex, S
3	4½ × 4	Small PF	Activated	Walls soft

Abbreviations: Th = thickness; Tex = texture; S = shape; Hol. S = hollow seed (integument only); PF = parthenocarpic fruit; * = ovules merely activated.

Analysis of the fruits was carried out as before. Controls were treated with lanolin, talc, water, and water and acetone. Most of the plants used were of strain No. 6 but a few flowers of strains Nos. 3 and 4 and La Paloma were treated.

Treatments with 2, 4-D in lanolin.—At concentrations of 1 p.p. 100,000 and 1 p.p. 10,000 there were no positive results. The largely positive effects of the higher concentrations are given in Table II.

Treatments with 2, 4-D in talc.—At concentrations of 1 p.p. 100,000 (eleven stigmas treated), 1 p.p. 10,000 (fourteen stigmas treated), and 1 p.p. 1,000 (ten stigmas treated), no activity was observed. Of the eleven flowers treated at 1 p.p. 100, three responded, while in the ten flowers of the "2 p.p. 100" class, two indicated positive results. Table III deals only with the five positive results obtained.

TABLE III

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
			2, 4-D in talc, 1 p.p. 100	
4	6½ × 4	PF	A few activated at top of column	Th, Tex, S
6	4½ × 2	Small PF	Activated	Th, Tex
6	3 × 1½	+	Active at tip of column	Th, Tex, upper ⅓ papery below
			2, 4-D in talc, 2 p.p. 100	
6	5 × 2½	Small PF	Upper ⅓ of column with small distorted Hol. S	Upper ⅔ Th, Tex, S, papery below
6	4 × 2	Small PF	Upper ⅓ of column active	Upper ½ Th, Tex, papery below

Treatments with 2, 4-D in water.—At 1 p.p. 100,000, twelve treated flowers gave no response. Three of eleven flowers treated at 1 p.p. 10,000, two of twelve flowers treated at 1 p.p. 1,000, one of eight flowers treated at 1 p.p. 100, and four of eleven flowers treated at 2 p.p. 100 gave positive results which are summarized in Table IV.

Concentrations of 1 and 2 p.p. 100 in lanolin gave by far the best results of the 2, 4-D treatments, but it seems unnecessary to go beyond 1 p.p. 100 (pl. 11, fig. 3). The resulting fruits ripened on the plant and split longitudinally, as do normal fruits, upon drying. They contained hollow "seeds"; that is to say no endosperm or embryo was present. These seeds are composed of ovular tissue, the integument, which apparently has been stimulated; they are normal in appearance except that they are usually smaller than true seeds and are often somewhat lighter

in color although they may be of characteristic darkness. The pattern of the normal seed coat is always apparent (pl. 12, fig. 3). There was no injury to plant parts through the lanolin mixture.

The poor results obtained with talc mixtures can probably be accounted for by the lack of solubility; apparently where positive results were obtained the stigma was unusually moist. No injury was manifest through talc treatments. The aqueous treatments, on the other hand, produced injury in twelve of the nineteen treated flowers in the classes 1 and 2 p.p. 100. Injury ranged from a single sepal with necrotic spots to complete browning of sepals and pedicel. There can be no doubt that injury is important in reducing the incidence rate of parthenocarpy in these groups. In addition to injury, another difficulty in using water as a medium is that it is extremely difficult, if not impossible, to confine the mixture to the stigmatic surface.

TABLE IV

Strain No.	Size (mm.)	Result	Ovules	Change in ovary walls
			1 p.p. 10,000	
4	5 × 4	Small PF	Activated	Th, Tex*
LaPal	3 × 1½	+	Slight activity	Upper ½ Th, Tex
LaPal	3½ × 2	+	Activity doubtful	Upper ½ Th, Tex
			1 p.p. 1,000	
LaPal	7 × 4	PF	Activated	Th, Tex, S
6	5 × 2	Small PF	Slight activity	Walls soft but capsule splitting*
			1 p.p. 100	
6	6¼ × 4½	PF	Hol. S	Th, Tex, S
			2 p.p. 100	
4	4 × 3	Small PF	Strong activity	Th, Tex
4	7 × 5	PF	Strong activity at top of column	Th, Tex, S
4	4 × 3	Small PF	No activity	Th, Tex
6	7 × 5	PF	Abundant Hol. S	Th, Tex, S

*Ovules merely activated.

PARTHENOCARPY IN X-RAYED OVARIES

Materials and methods.—No. 6 plants were supported so that the flowers rested on a ring covered with Scotch tape. The flowers were strapped in place with Scotch tape on either side of the ovary, care being taken to center the ovary under the target. The technical factors were target distance 15 cm., filter ½ mm. of aluminum, 120 KV, 10 milliamps, H.V.L. = 1.6 mm. of aluminum. Ovaries were treated with 2400, 3000, 3600, 4200, 4800 and 5400 r; the number of fruits

harvested at maturity in each dosage class was 12, 7, 7, 7, 12 and 7, respectively. Untreated pollen from La Paloma flowers was used; contamination after pollination was prevented.

Results.—There was considerable variation in the effect of radiation on the ovule as far as seed development was concerned. In the "2400 r" class fruits contained filled seeds, partially filled seeds, empty but normal-appearing seeds, highly distorted empty seeds, and ovules showing only signs of initial development. Low levels of ovule activation are difficult to assess because there is no way as yet to determine whether an ovule is arrested in development because of radiation damage or whether it simply did not receive enough growth substance following pollination.

Only two fruits in the "3000 r" class contained some filled, partly filled, and round empty seeds. The remainder contained highly distorted ovular structures and ovules indicating little or no activation.

Three fruits of the "3600 r" class contained some filled, partially filled, and empty seeds. Some of these seeds were found to contain a soft, milky material. The remainder contained highly distorted empty seeds and activated or inactivated ovules (pl. 12, fig. 4).

The fruits of the remaining classes (4200, 4800, and 5400 r) contained only distorted empty seeds and ovules at various stages of activation.

Table V gives the results of a germination test conducted in constant illumination of 100 foot-candles supplied by fluorescent "daylight" bulbs and temperature of 25° C. Seeds were sterilized in 3 per cent hydrogen peroxide and germinated in Petri plates on filter-paper moistened with Vickery's solution. Counts were made eleven days after sowing. A germination test is hardly a suitable index of x-ray damage since seeds that germinate may give rise to seedlings that die somewhat later. Furthermore, this test cannot be regarded as definitive because of the small number of seeds per sample.

TABLE V

Dosage r	Seed number per sample	Sample wt. (mg.)	Full germination to 2 cotyledons	Laggards	Total
2400	100	7.29	7	8	15
3000	100	6.39	5	3	8
3600	75	4.22	2	3	5
Control	100	11.48	24	23	47

Conclusions.—Treatment with 2400 r is often fatal to egg and polar nuclei. Many fruits in this class contained a large number of empty as well as filled seeds, indicating that often the integument alone had proceeded to final development. The empty seeds are frequently quite normal in appearance and difficult to distinguish from filled seeds. The integument thus appears more resistant to treatment by x-rays than the internal tissues of the ovule.

The crumpled appearance of the distorted empty seeds which occur in all classes might be taken as an indication of radiation damage to the integument rather than evidence of collapse of the internal tissues of the ovule. Yet empty seeds with the same degree of distortion are found when irradiated pollen is placed on the stigmas of untreated flowers. In this case the integument has not been treated and the subsequent distortion must be due primarily to collapse of internal ovular structure. The integument may suffer injury but it is difficult to distinguish between damaged and collapsed integument.

The ovary wall and placental column are more resistant to x-radiation than the other tissues of the ovary. The walls develop the texture, thickness, and shape of normal fruits and split at maturity even under large doses (pl. 11, fig. 4).

EFFECTS INDUCED WITH IRRADIATED POLLEN

Materials and Methods.—Mature pollen from shattered anthers of La Paloma flowers was gathered and placed in No. 2 gelatin capsules prior to radiation. The technical factors involved were the same as for the irradiation of ovaries. The capsules were held in place on the ring with small strips of Scotch tape. Following treatment the pollen was placed on the stigmatic surfaces of No. 6 flowers. Protection against undesirable pollination was provided. There were in all fourteen radiation classes. Beginning with 13,200 r and increasing at increments of 600 r, the treatments were carried on until a dosage of 18,000 r was reached. They were resumed at 20,000 r, and the following doses were given: 22,200, 23,400, 24,600 and 25,800 r. Eight to ten fruits were analyzed in each class.

Results: Classes 13,200 r to 17,400 r.—The seed set was abundant. In the classes through 16,800 the completely filled seeds exceeded the partially filled and empty seeds although this excess appeared to decrease as the dosage rose. There was a steady increase also in the number of ovules giving rise to flat, cup-shaped and distorted structures, indicating damage to male nuclei. In class 17,400 the filled seeds were about equal to the partially filled and empty seeds.

Class 18,000 r.—Seed was generally abundant, with filled seed equalling partially filled to empty seed in about half the fruits. In the remainder, the partially filled to empty seeds exceeded the filled. An increase in the number of flat, cup-shaped, and distorted structures arising from ovules activated to a somewhat lesser degree was apparent.

Class 20,000 r.—Filled seed appeared to be about equal to partially filled and empty seed. Large numbers of distorted ovular structures and ovules that had been merely activated were observed, indicating that an increasing number of ovules was receiving badly damaged male nuclei or was simply undergoing a purely chemical activation.

Classes 22,200 to 25,800 r.—In these groups there were fewer filled seeds than partially filled and empty seeds. The number of distorted ovular structures is far greater than the number of recognizable "seeds," whether empty or filled, indicating that most of the male nuclei have undergone damage (pl. 12, fig. 5).

The difficulty in measuring precisely the amount of damage sustained by the pollen grain is apparent in the following comparison. In class 23,400 r, nine fruits were analyzed. The average number of recognizable "seeds" (filled, partially filled, or empty) was 29 per capsule. The ratio of filled seeds to partially filled or empty seeds at one extreme was 1 to 9, at the other 0 to 50. The average was 1 to 36. Normal fruits of this size might contain from 100 to 250 viable seeds. The large number of ovules that had undergone stimulation but had failed to develop would therefore indicate a high degree of damage.

In class 25,800 r ten fruits were analyzed. The average number of recognizable seeds per capsule was 57.4, higher than in class 23,400, although again the number of ovules falling short of complete development is high when compared with the number of seeds occurring in a normal fruit. The ratio of filled to empty or partially empty seeds was 1 to 4.6. Although this is an extreme case, it illustrates the difficulty in giving a true evaluation of damage. Variation in the number of seeds, damaged or otherwise, or in the number of activated ovules, might be due in part to the number of pollen grains employed. However, it is more likely due to the degree of damage suffered by the pollen grain depending upon where and how it is hit.

In general, it seems safe to say that with increasing dosage, fewer filled seeds are developed, that the number of partially filled and empty seeds increases, and that finally the number of ovules merely undergoing some degree of activation increases. It would appear from examination of large numbers of activated ovules and completely hollow "seeds" composed only of integument that x-radiation of pollen grains, severe enough to kill nuclei, often does not nullify the stimulating effect of the activating substances or their precursors within the grains. The growth or activating components of the grains retain some ability to stimulate the ovary wall as well as the integument so that sometimes these fruits are of much the same size as a normal fruit (pl. 11, fig. 5).

The following tables indicate that no completely parthenocarpic fruits have been derived from x-rayed mature pollen, since even in the higher dosage classes, seeds capable of germination developed. Rick (1943), treating *Petunia* anthers immediately prior to anthesis, found that dosages as high as 50,000 r (200 KV, 10 ma, filters of ¼ mm. copper and ¼ mm. aluminum, target distance 10 cm., Wappler clinical unit) permitted the production of viable seed.

Germination test No. 1 was carried on under greenhouse conditions, the counts being made three weeks after sowing the seeds on moist filter-paper, following sterilization with Sarasan. Germination test No. 2 was carried on under the conditions described on page 103.

TABLE VI

GERMINATION TEST NO. 1 (Samples 100 seeds each)

Dosage r	Full germination to 2 cotyledons	Laggards	Total
Control	48	15	63
13,200	35	5	40
13,800	36	8	44
14,400	38	7	45
15,000	26	12	38
15,600	32	10	42
16,200	12	13	25
16,800	19	5	24
17,400	14	11	25
18,000	13	5	18
20,000	22	8	30
22,200	0	1	1
23,400	0	0	0
24,600	0	0	0
25,800	10	7	17

GERMINATION TEST NO. 2

Dosage r	Seed number per sample	Sample wt. (mg.)	Full germination to 2 cotyledons	Laggards	Total
13,200	100	7.20	21	10	31
13,800	100	7.03	20	20	40
14,400	100	8.44	19	16	35
15,000	100	7.00	20	18	38
15,600	99	8.24	20	15	35
16,200	100	7.04	6	6	12
16,800	100	6.64	19	7	26
17,400	100	7.29	4	12	16
18,000	100	5.83	9	4	13
20,000	100	6.40	11	5	16
22,200	100	4.65	2	1	3
23,400	55	2.23	0	0	0
24,600	55	1.85	0	0	0
25,800	100	5.41	6	2	8

PARTHENOCARPY INDUCED WITH POLLEN FROM X-RAYED ANTHERS

Since pollen grains collected at anthesis require such high dosages for inactivation, anthers were taken from La Paloma flowers about to open. At this time anthers contain pollen but are plump and juicy.

Materials and Methods.—The anthers were placed in a No. 2 capsule, irradiated, and then allowed to ripen and shatter within the capsule. The pollen was then applied to No. 6 flowers, with soda straws being used to prevent any additional pollination. The x-ray doses ranged from 5400 r to 13,800 r at increments of 1200 r. Only the most turgid anthers from a single flower were treated in each class because occasionally one anther may be non-functional.

Results.—Classes 5400 and 6600 r were the only ones in which any filled seeds were found (pl. 12, fig. 6). Variation in the effectiveness of radiation was apparent in these two groups; only one fruit in the "5400 r" class contained any filled seed while in all three of the "6600 r" group a few were found. No filled seeds were found in the remaining classes except in the "12,600 r" class where one fruit contained one filled seed. In general, it may be said that as the dosage increased, the number of empty seeds increased until the bulk of the ovules was merely in some stage of activation, some remaining completely unstimulated.

In the "12,600 r" class the ovary walls did not develop completely but remained papery at the base. The upper portions showed characteristic texture, thickness, and shape. These fruits were also the smallest obtained in addition to containing the least activated ovules. In this experiment there appears to be a decrease in the size of the fruit with increasing dosage, indicating injury to the pollen growth substances or their precursors.

None of the four flowers treated with 13,800 r pollen developed. This is not surprising in view of the effects of increasing dosage on fruit development. The fact that the flowers treated with pollen receiving a dosage of 10,200 r failed to develop either indicates variability in response or else that other factors were involved (pl. 11, fig. 6).

DISCUSSION

Murneek (1951) has concluded that synthetic growth substances are not in themselves always responsible for fruit development but rather that they stimulate in some fashion a hormone or hormones already present in female tissue. This view is not too far removed from that taken by many investigators with regard to the activity of pollen; that is to say, that the activity of pollen, aside from furnishing nuclei in the formation of embryo and endosperm, is based on a substance which sets into motion a hormone system resulting in ovary enlargement.

There are ample references to the hormone content of pollen grains in the literature of plant growth substances, and Muir (1951) sums up the situation when he states that pollen of all sorts probably contains auxin, but that it may vary in amount and in condition; auxin may exist in a free or bound condition or as a precursor, and failure to detect it has been due to faulty techniques. Wittwer (1951) contends, as has van Overbeek et al. (1941), that in an actual pollination the number of grains involved is too small to furnish adequate hormone material for fruit production. Muir's (1947) experiments are of particular interest here because of the relationship between *Nicotiana* and *Petunia*. Pollen of *N. Tabacum* was found to contain only small amounts of free hormone with somewhat larger quantities in the bound condition. The unpollinated pistil indicated no free hormone, but considerable hormone in the bound state. A water extract of pollen was found to release much larger quantities of bound hormone in the free condition from dried ovary tissue. In a later report (1951) he estimated

that following fertilization the auxin content in the ovary is 100 times greater than the maximum amount obtained from extraction of pollen. It was 30 times greater in the style. It would appear, then, that there is something in pollen other than its native hormone complement which instigates the release of hormones in the ovary following pollination. After fertilization the ovules become a rich source of hormones as indicated by the experiments of Wittwer (1943), Britten (1950), and others.

The development of integument and ovary wall need not in certain cases be dependent upon the development of endosperm and embryo. Studies with 2, 4-D and other substances have resulted in the production of parthenocarpic fruits filled with empty seeds. The use of foreign pollen, as shown here, occasionally results in parthenocarpic fruits containing empty seeds, the emptiness apparently due to genetic differences between sperm and egg, while the seed coat and ovary wall are stimulated by the less specific activators within the grain. Furthermore, pollen grains treated with x-ray dosages sufficient to render their nuclei genetically inactive, can still stimulate integument and wall growth although it has not been determined histologically as yet that fertilization followed by collapse of the system within the integument has not occurred. This last point deserves amplification. A glance at the data concerning the fruits produced with irradiated dry pollen shows that none of these was completely parthenocarpic. Even in the highest dosage class a few filled seeds developed and there were others partially filled. Since fertilized ovules are known to be rich sources of hormone, it is easy to visualize a diffusion of hormone material from fertilized to adjacent unfertilized ovules with the subsequent expansion of integument and wall tissues. Britten (1950), studying maize, concluded that naturally parthenocarpic fruits resulted from the activity of auxin products emanating from seeds developing close by. The spatial arrangement of parthenocarpic and normal fruits on the ear coincided with vascular supply. In these *Petunia* fruits, it would seem possible, even when male nuclei had been damaged, for fertilization to occur, and, providing that collapse of the fertilized egg apparatus did not take place too soon, a diffusion of hormones could begin. In the cases of parthenocarpic fruits produced by irradiating ovaries or turgid anthers, this does not appear to be as important a consideration, since the appearance of the integument indicates a very early collapse of the nucellus and they are usually completely empty. Radiation damage to the male nuclei had apparently been severe enough to prevent fertilization.

Whether integument can develop to any extent without development of the ovary wall remains to be seen. Some treatments in this study with 2, 4-D in lanolin at 1 p.p. 1,000 and at various concentrations in talc (when moisture was present) have resulted in small parthenocarpic fruits in which the only activated integuments were located on parts of the placental column opposite wall tissue showing normal thickening and texture. Those ovules opposite less-developed portions of the ovary wall such as the bases of these small fruits, which usually

remain thin and papery, showed little if any activity. To activate integument separately, an activator not stimulating other tissues would be necessary, and whether the space required for enlargement would be available without growth of the wall seems doubtful.

Since ovary walls and ovules can, under certain conditions, act independently, then 2, 4-D, when applied to the stigma of *Petunia*, is usually an activator for both systems. X-rayed pollen and the pollen of *Nicotiana affinis* would appear to be in the same category.

If we assume that it is possible for all pollen types to have within them certain activating substances in common but that the pollens of genetically related groups exhibit fewer and lesser differences among themselves, then it is possible to account for parthenocarpy arising as it does here from a combination of solanaceous pollen and *Petunia* stigmas. It is then possible to account also for the exceedingly mild activation provoked by the orchid pollen in *Petunia* ovaries. Such an explanation would require that basically similar pollen grains produce, or do not produce, results depending upon the orientation of these substances in a genetically suitable background. In short, they must find the proper kind of stigma. That nuclei involved in fertilization have a much stricter limitation placed upon them has been amply illustrated in the failures of numerous attempts to obtain seeds from certain interspecific or intergeneric crosses.

The activating substance or substances in pollen seems to be independent of the nucleus, in a functional sense at least, at the time of pollination, since parthenocarpic fruits tend to be produced by irradiated pollen although pollen nuclei have been damaged by x-rays. The substance appears to be more stable in the presence of x-rays than the nucleus. This stability is not as great when turgid anthers are irradiated as when dry pollen is treated as indicated by fruit size, and it is possible that such resistance varies with moisture content (Lea, 1947). The nuclei of dry pollen too require higher lethal doses than those in the moist anther, but here the question is further complicated in that the nuclei of dry grains are further removed in time from completion of meiosis than the nuclei of less mature grains.

SUMMARY

1. Fourteen types of pollen were placed on the stigmas of 349 *Petunia* flowers. Five of these pollen types were solanaceous, two of them (*Nicotiana affinis* and *Salpiglossis* sp.) producing parthenocarpic fruits which were somewhat smaller than normal fruits.

2. Parthenocarpic fruits have been produced in *Petunia* with 2, 4-D, x-rayed pollen, and x-rayed ovaries.

3. The effects of these methods are discussed with regard to fruit development.

4. The lethal dose for egg and accessory cells appears to be from 2400 to 3000 r under conditions outlined above. Completely lethal doses for nuclei of

moist pollen (in the anther) were about 6600 r and for dry pollen undetermined, but over 25,800 r. The ovary wall, the integument, and the placental tissue, perhaps because of their relative dryness, showed no ill effects from treatments up to 5400 r and responded normally to activating substances of pollen. The activator substances of pollen grains require a higher lethal dose than nuclei in both dry and moist pollen, although in treating moist pollen (in the turgid anther) these lethal doses are lower.

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Explanation of Plates

PLATE 11—FRUITS

Fig. 1. Control (left); *Cymbidium* male × *Petunia* female (note activation in upper half); normal *Petunia* fruit at right.

Fig. 2. Three parthenocarpic fruits from the cross *Nicotiana affinis* male × *Petunia* female. Normal *Petunia* fruit at right.

Fig. 3. Control; 2, 4-D in lanolin, 1 p.p. 1,000; 2, 4-D in lanolin 1 p.p. 100; normal.

Fig. 4. Six fruits from x-rayed ovaries treated with normal pollen. (2,400; 3,000; 3,600; 4,200; 4,800; 5,400 r). Normal fruit at right.

Fig. 5. Two fruits resulting from pollen treated with 25,800 r applied to normal flower. Normal fruit at right.

Fig. 6. Six fruits resulting from pollen treated before anthesis and applied to normal flowers. The largest fruit from each dosage class is shown here (5,400; 6,600; 7,800; 9,000; 11,400; 12,600 r). Normal fruit at right.

PLATE 12—SEEDS

Fig. 1. Normal seeds, × about 5.33.

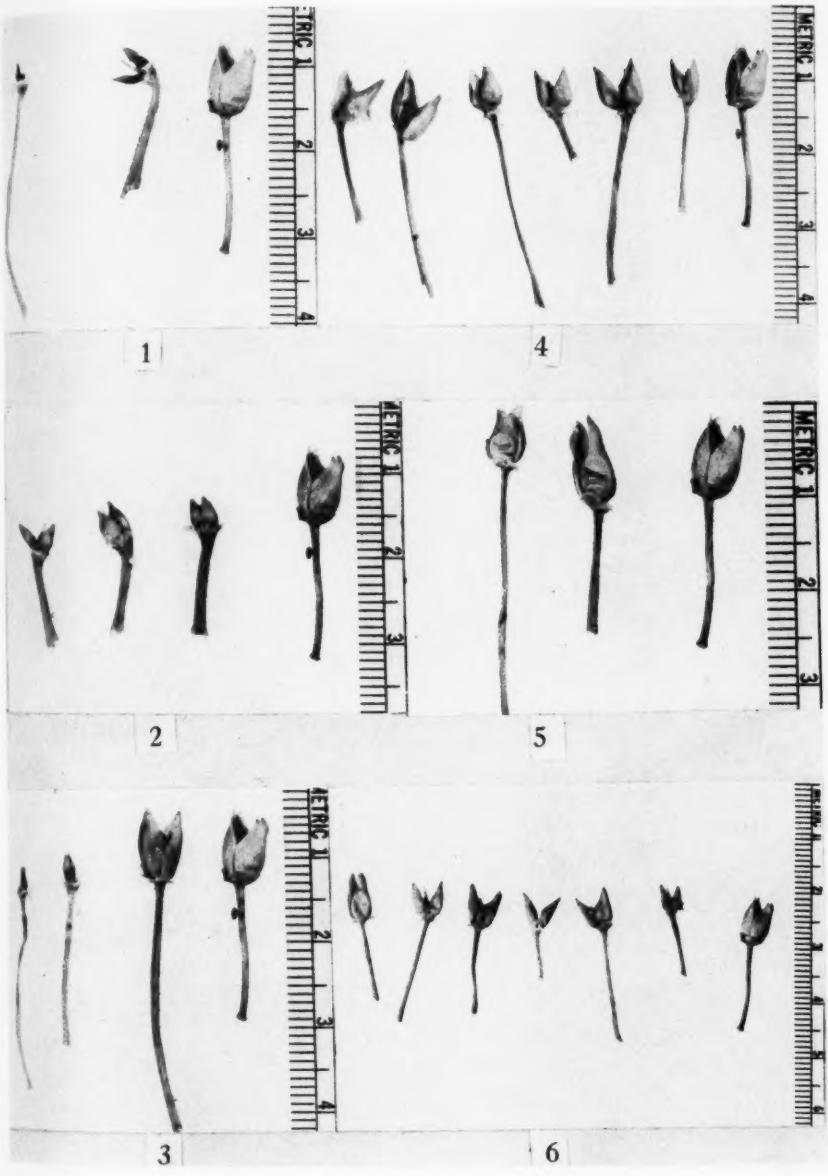
Fig. 2. Hollow seeds of *Nicotiana affinis* male × *Petunia* female, × about 5.33.

Fig. 3. 2, 4-D in lanolin, 1 p.p. 100, × about 5.33. Some crushed seeds have been added to show the hollow condition.

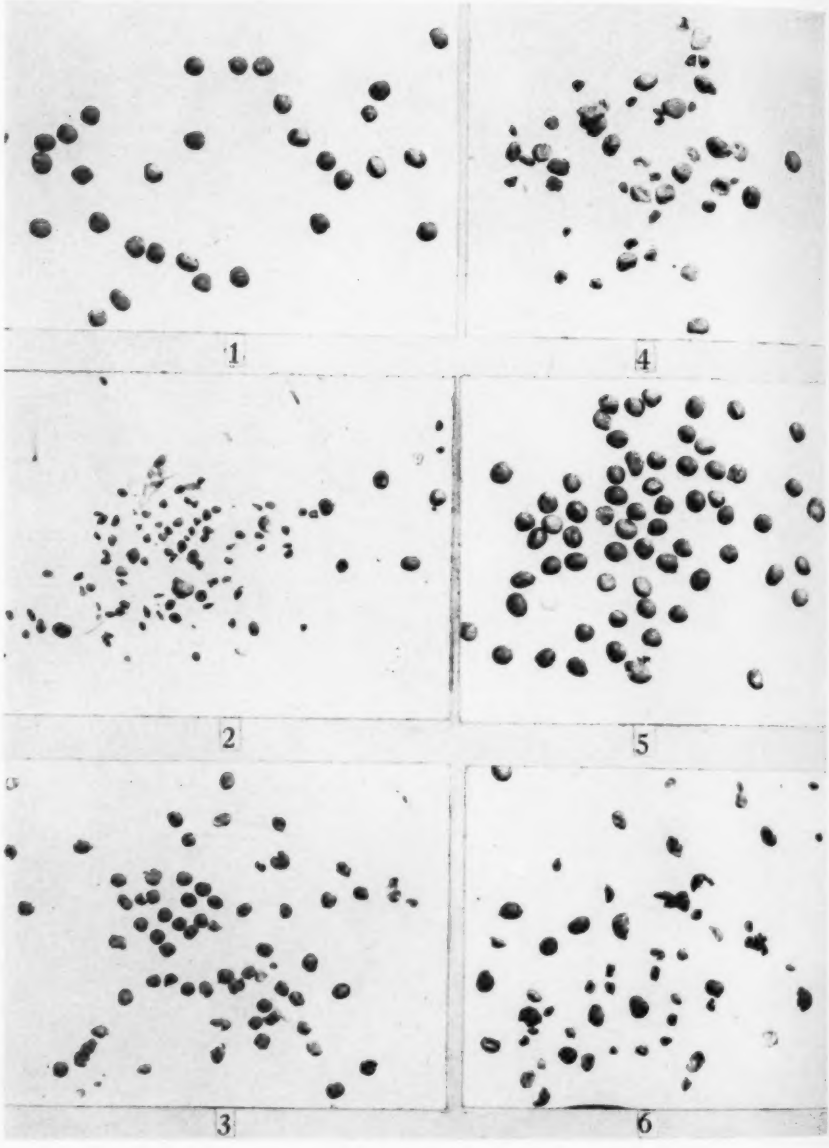
Fig. 4. Hollow seeds from ovaries treated with 3600 r × normal pollen, × about 5.33.

Fig. 5. Seeds from pollen treated with 25,800 r × normal flowers, × about 5.33. Some crushed seeds have been added to show the hollow condition.

Fig. 6. Seeds from normal ovaries × pollen from anthers irradiated at 5,400 r prior to anthesis, × about 5.33.



McQUADE—PARTHENOCARPY IN PETUNIA



McQUADE—PARTHENOCARPY IN PETUNIA

A GEOGRAPHY OF POKEWEED*

JONATHAN D. SAUER**

The plants we call weeds stand apart from their truly wild and truly tame fellows because of their special ability to establish themselves in artificial habitats. In spite of indifference or active repression by man, they have been able to thrive and multiply with the advance of civilization. By the very fact of their existence such plants suggest problems of special botanical and ethnological interest. There is the problem of the peculiar characteristics which have allowed the weeds to exploit disturbed places. There are also the questions of how the ancestors of modern weeds fitted into the ancient natural plant associations of pre-human times, how much these plants have evolved, and how far they have migrated since they first allied themselves with man.

General answers to such questions will require understanding of the stories of many individual species. Since only fragments of direct historical evidence on most weed species can be found in published records or herbarium collections, their stories must be reconstructed largely from indirect evidence. One of the most powerful lines of indirect evidence may be found in geographic distributions. The present geographic patterns of the weeds, like those of any phenomena irregularly distributed over the earth's surface, offer strong though sometimes complex and cryptic clues to their past stories.

This paper represents an attempt to describe and understand the distribution patterns of a single species, *Phytolacca americana* L. (= *P. decandra* L.; includes *P. rigida* Small), commonly called pokeweed or simply poke. Poke is in some ways an especially attractive subject for such a case study. The species is relatively clear-cut taxonomically and is the sole representative of its genus through almost its entire range. Thus a wealth of previous records can be used in studying its distribution, with slight danger of accepting mistaken identifications.

The gross range is considered first, followed by examination of the micro-distribution. Finally, an effort is made to reconstruct some of the story of how poke became a successful weed.

Gross Range

NATIVE RANGE.—

Like most of the species of *Phytolacca*, poke is a native of the New World. Unlike all the other New World species, poke has its range centered north of the tropics. Its native area presumably includes a little of southeastern Canada, almost the entire eastern half of the United States, and a small area in the extreme northeast of Mexico. The northernmost outposts of a few tropical species reach into the Bahamas and northern Mexico. There they approach the southernmost

* An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University.

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poke colonies of Florida and Texas; in northeastern Mexico there is some actual overlap of ranges. I have seen specimens of both typical *P. americana* and of tropical *Phytolacca* species which were collected at Monterrey in Nuevo León.[1]

The first available historical records of poke were made during colonial times in New England, Pennsylvania, and Virginia (Parkinson, 1640; Benson, 1937). Before 1850 poke was reported in Kansas (Townsend, 1839), close to its present western limit. Not until late in the nineteenth century are there enough historical records to give even a rough outline of the range of the species. By that time the increasingly adequate published floras and herbarium collections indicated a range approximating that of the present day, including such border-line areas as Ontario, Wisconsin, Minnesota, Iowa, Nebraska, Oklahoma, and Texas.

The available recent records of poke in eastern North America are mapped in fig. 1. The indicated locations are based partly on published local floras, too numerous to list here, partly on herbarium specimens I have examined, but mostly on private records and summaries of herbarium collections which were supplied by the botanists named in the acknowledgments. Special efforts were made to obtain adequate data from the states on the western and northern margins of the range, and the localization of dots within those states is probably significant. In the southeastern states the specific locations of the dots and their low densities probably signify nothing more than inadequate records. In spite of the scant records, there is reason to believe that poke is abundantly and generally distributed throughout the Southeast. In Arkansas, for example, "the plant is in every section of land in the state," according to Demaree,[2] while in Georgia poke has been seen in "more than 100 counties" by Cronquist.

At the present time poke seems to have a coherent distribution blanketing most of the eastern United States. Within the general limits of its range, large-scale gaps in the actual distribution may be found only in mountain areas. Toward the northern limit poke is reported only from very low elevations. As far south as New York State, it is infrequent at elevations above 1,000 feet. Figure 2 shows the available records of poke in New York, based mostly on unpublished data from the New York State Museum and the New York Botanical Garden, supplied by S. J. Smith. Contour lines are drawn according to Rafter (1905). In the more

[1] A few specimens of *Phytolacca* collected far to the south in Vera Cruz have been described by Walter (1909) as representing a variety of *P. americana*. Those specimens cited by Walter which are available to me resemble but are by no means identical with *P. americana* proper. The Vera Cruz colonies may be, as Walter assumed, simply aberrant and isolated tropical outposts of the species proper. However, they may be of hybrid origin. In key characters, including carpel and stamen number, *P. americana* is intermediate between certain tropical species which range into Vera Cruz. These species are known to hybridize elsewhere in tropical America, and some individual segregates from such crosses have the key characters of *P. americana* (Fassett and Sauer, 1950). I doubt if the taxonomic position of the specimens in question can be positively determined without much better samples of the *Phytolacca* populations of northeastern Mexico.

[2] Complete names and addresses of persons supplying unpublished data are given in the acknowledgments.

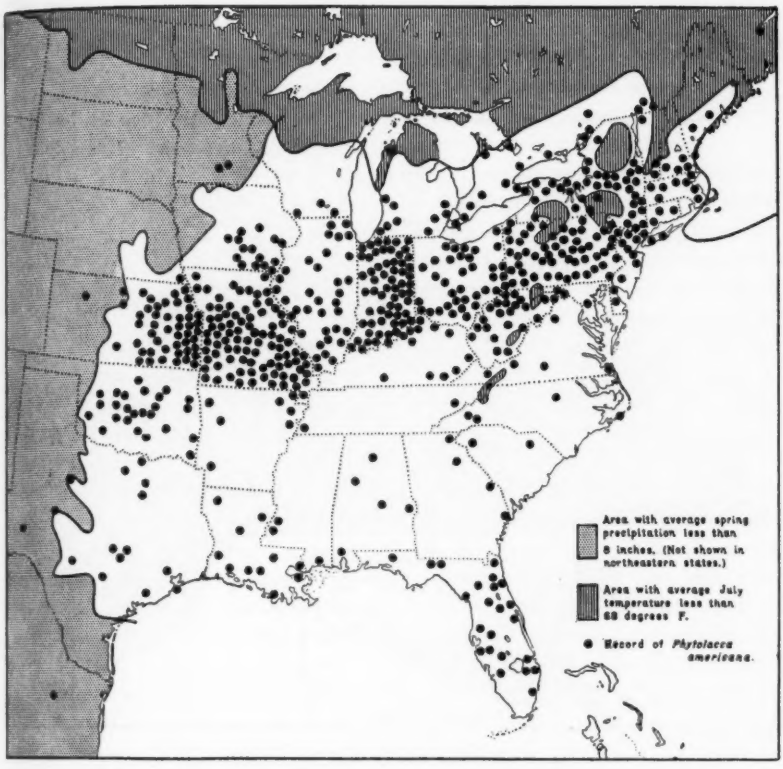

Fig. 1. Distribution of poke in eastern North America.

southern states poke may be absent only on the highest mountains; it has been found at elevations up to 2,000 feet in Pennsylvania, 2,500 feet in West Virginia, 4,000 feet in Virginia, 3,000 feet in Tennessee, and 2,800 feet in Arkansas.

The apparent absence of poke in some highland areas and the general east-west trend of the northern limit of the species suggest that its northward extent may be determined by temperature tolerances. Rough correlations can be made with various measures of temperature. For example, the plant has seldom been found in areas where the temperature falls below —20° F. in an average winter, so that winter-killing of the ordinarily perennial roots may sometimes be a limiting factor. However, the range can be correlated more closely with summer than with winter temperatures, and there is some experimental proof that summer temperatures are critical.

Lloyd (1914, 1917) planted *P. americana* seeds of unspecified origin at Carmel, California. He found that poke germinated and grew normally there, but pro-

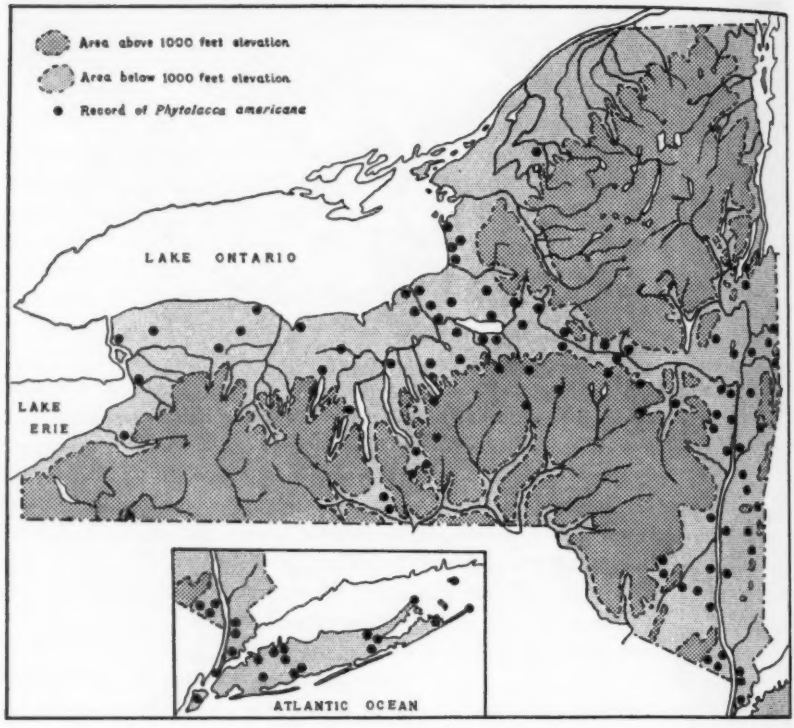

Fig. 2. Distribution of poke in New York State.

duced only abortive flowers when grown in the open. Plants exposed to slightly higher temperatures by being planted against a sunny wall, prostrated against the ground, or grown in an unheated, well-ventilated glass shelter, flowered normally and produced viable seed. Lloyd concluded that prevailing daytime temperatures at Carmel were about 5° F. below the critical level for seed production. During the period of his observations, the daytime temperature usually ranged between 60° and 70° F., exceeding 70° on less than a third of the days.

Although the precise limits of the northward extent of the species probably involve a complex balance of factors, it seems reasonable that the major control may be duration of temperatures above the minimum required for flowering and seed set. Since adequate temperature data in terms of durations are not available, a correlation can be attempted only with average temperatures. As shown in fig. 1, the line dividing zones with an average July temperature of over 68° F. from cooler zones approximates the northern boundary of the species fairly well.[3]

[3]All climatic data used in this paper are from Brooks and Ward (1936) and Kincer (1941).

The position of the 68° July isotherm in mountain areas also corresponds fairly well to the upper altitude limit of poke. In the New York area, this isotherm follows the 1,000-foot contour line, shown in fig. 2, rather closely, while farther south July temperatures average above 68° in all but the highest mountains.

The westward extent of poke appears to be limited by moisture rather than temperature. The western border, lying more or less along the 100th meridian in a zone of sharply decreasing precipitation, can be roughly correlated with various measures of moisture. Here again, the exact limitation of the range is probably controlled by a complex balance of factors, including water supply and transpiration rate during different stages in the life history of the plant. However, experience in growing poke plants indicates that moisture is most likely to be critical during the young seedling stage. Mature plants with their well-developed fleshy roots are much better able to stand drought. It seems reasonable that on the Great Plains border, rainfall during the seedling stage should be a major limiting factor. The line dividing zones with an average spring rainfall over 8 inches from drier zones approximates the western limit of the species fairly well, as shown in fig. 1. In the dry plains west of this line poke is very rare and is confined to peculiarly moist habitats, such as river bottoms. The plant becomes abundant in upland habitats only where spring rainfall exceeds 10 inches.

Thus it appears that, within its native area, the general distribution of poke is largely controlled by its climatic tolerances. Although poke has a long history of human use throughout this area (Sauer, 1950), man has ordinarily been satisfied with gathering the spontaneous supply of the plants. Deliberate propagation has certainly been attempted in isolated instances, but I know of no evidence that these efforts have in any way affected the gross range of poke in eastern North America.

AREAS OF RECENT INTRODUCTION.—

Outside its native area, poke owes a great deal of its distribution to human appreciation of its useful properties. Poke's most conspicuously successful colonization abroad is in the Mediterranean region, where it was introduced about 1650. Its berries proved so useful for coloring low-grade wines that the plant became widely cultivated in Portugal, Spain, France, and Italy (Ascherson and Graebner, 1915; Messedaglia, 1927). Escaping from cultivation, poke has become a fairly common weed in this region. It is reported from almost all the European and African countries bordering the Mediterranean Sea, and ranges northward into Switzerland, southern Germany, Austria, Hungary, and Russia, eastward to Persia, and westward to the Azores, Canaries, and Cape Verdes (Walter, 1909; Hegi, ca. 1910; Ascherson and Graebner, 1915; also specimens in the Missouri Botanical Garden Herbarium). Poke has occasionally been planted as an ornamental in some European countries, including England and France (Saint-Hilaire, 1809; Weathers, 1901). A form with variegated foliage, propagated by root division, was once sold commercially in Paris (Carrière, 1887).

The violent drug properties of poke seem to have had little to do with its propagation in Europe. It is mentioned in very few of the European books on medicinal plants. However, introduction of the species elsewhere may be traceable to its drug effects. Poke is reported to have been brought to Cuba as a cultivated medicinal plant (Roig y Mesa, 1945). Introduced into South Africa, poke has naturalized itself as a weed around settlements; the juice of the berries is used for coloring food and beverages, while the roots are used as medicine by the Kaffirs (Marloth, 1913; Watt and Breyer-Brandwijk, 1943). The species is also reported as an adventitious plant in such widely scattered areas as California, Arizona, Bermuda, Asia, Australia, and Macronesia, but no details are available (Walter, 1909; Ascherson and Graebner, 1915; Britton, 1918; Robbins, 1940; Kearney and Peebles, 1942).

MICRO-DISTRIBUTION

A brief inspection of records of occurrence of the species or a casual look at the plants in nature shows immediately that poke has a peculiarly spotty distribution pattern throughout its range. The poke population, even near the heart of its native area, is made up of solitary individuals and scattered colonies, all closely associated with disturbed habitats. Thus poke presents a curious picture of a plant which behaves like an immigrant weed, even in its homeland, and seems to occupy a niche in no natural plant association.

FIELD STUDY OF MICRO-DISTRIBUTION.—

In an effort to understand the peculiar distribution pattern of poke, a small area in the heart of the native range of the species was selected for detailed field study. The sample area, shown in fig. 3, is part of the Missouri Botanical Garden Arboretum at Gray Summit, 35 miles west of St. Louis. It covers a narrow strip about one mile long, running from the alluvial Meramec River bottoms up over rocky bluffs with limestone outcrops, to a rolling upland capped with silt loam. The vegetation cover is highly varied, including cultivated fields, pasture, former farmyards, natural glades, and woodlands of many different ages and compositions, but with oaks, maples, and hickories predominating. All the poke plants that could be found in this area in the early fall of 1947 are mapped on fig. 3.

Upland colonies.—About forty plants were growing along a dry gully in the northwest corner of the area. Most of these were mature plants, scattered through the brush on the gully sides; some young seedlings had come up where brush had been recently cut. In the open grassland at the very head of this channel, there was a small group of seedlings where the turf had been cut by running water. There were several more upland colonies near the road in the north-central part of the area. Most of these plants were either actually on the sites of former farm buildings or close by in an old mule yard, kitchen garden, and similar areas of once intensive human activity. According to the records these buildings were torn down at different times between 1938 and 1942. For the last few years the

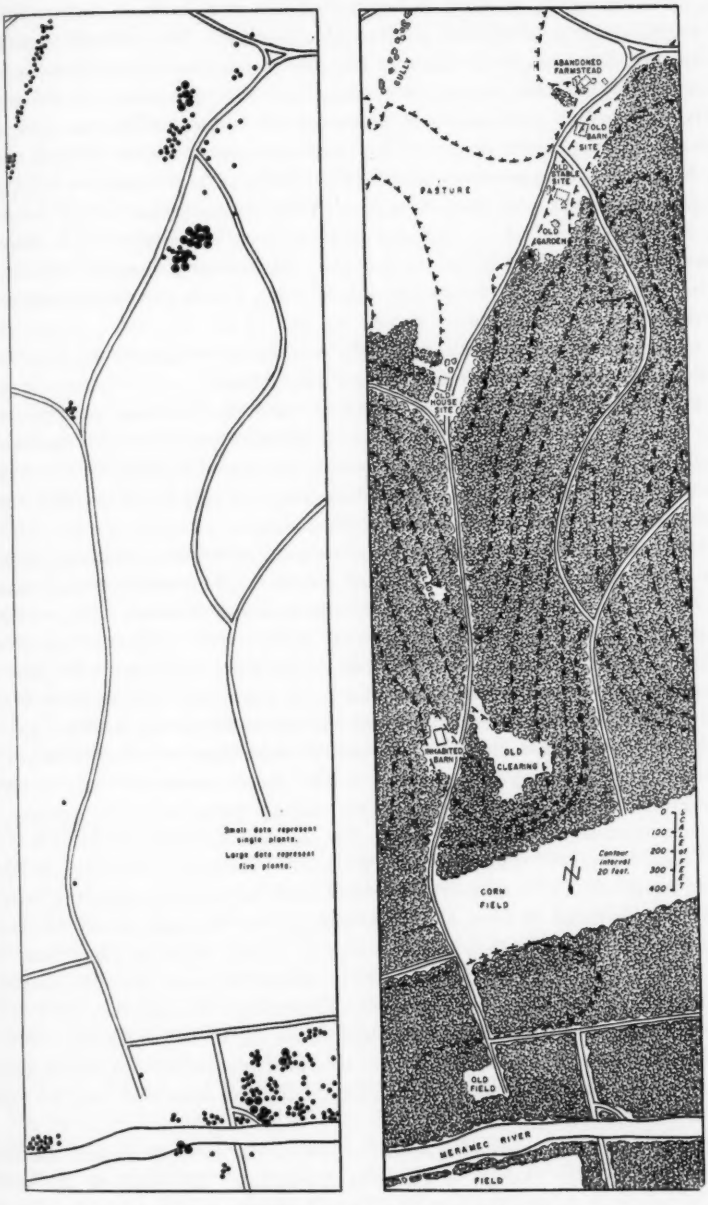

Fig. 3. Distribution of poke in area of field study.

area had been lightly pastured and mowed almost every summer, and at the time of mapping was covered with a heavy bluegrass sod. No seedlings were found in the unbroken grassy sod. Most of the poke plants were quite old and appeared to be hanging on from past periods of disturbance. From each of the tremendous, profusely branched perennial roots, sometimes over a foot in diameter, grew large numbers of short slender stems. Leaves and inflorescences were relatively small.

Numerous seedlings surrounded a newly fallen tree at the south end of the old kitchen garden and were scattered over a fresh excavation near the old farmhouse site at the north end of the area. A single seedling was coming up in the fresh gravel of a road behind the old stable site. These younger plants had only one or two stems from each slender root; their stems, leaves, and inflorescences were relatively large.

Except for two solitary and unhealthy looking individuals, no poke was found between the old upland farm sites and the river bottoms.

River-bottom colonies.—There had been some recent human activity in the river bottoms also. The south bank had been partially logged two years previously; on the north bank small-scale timber cutting and gravel digging were in progress during the year of mapping. Flourishing poke colonies were crowded around brush piles and felled trees in these disturbed places.

Many other river-bottom colonies, including abundant seedlings, occupied sites where there was no trace of human activity. They were scattered through the more open river-bank woods, most often among sycamores, but sometimes among hackberry, soft maple, cottonwood, or elm trees. All of these colonies occupied places where there was very little low-growing vegetation. No poke was found in mature woods where there was a heavy growth of other herbs or in cut-over areas with a dense stand of brush or young second-growth timber.

Although there was no sign that man had ever disturbed many of the river-bottom sites occupied by poke colonies, another factor causes repeated and violent disturbance of this habitat. During an average spring or early summer, the Meramec leaves its banks at least once. The poke colonies lie in the zone of maximum flood frequency. Piles of river drift, beds of fresh sand and other alluvium, caving banks, and raw cuts give good evidence of the powerful disruption effected by the river. Roots of some of the old poke plants had been almost completely exposed; these plants were stunted, sometimes dying, although many were still able to bloom and fruit. Where roots were covered by fresh alluvium, sometimes as much as two feet deep, the plants were flourishing. Among these were found the largest plants in the area mapped, some measuring 12 feet from root crown to branch tip, bearing enormous leaves, some of which approached two feet in length. The stout, sparsely-branched tap-roots plunged straight downward into the sandy soil for more feet than I cared to dig.

The colonies three years later.—In the summer of 1950, I revisited the same area to look for any changes in the poke population. The gully in the northwestern corner of the area had been filled in by bulldozers and was heavily trampled

by cattle coming to drink at a newly constructed pond. The poke colonies in that part of the area were completely obliterated. Along the road on the other side of the same pasture, about a dozen plants remained of the more than fifty which had been present in 1947. The poke plants, though seldom eaten by cattle except when very young, had suffered from the increased cow traffic around the new pond. The site of the largest colony had been scuffed completely bare, and some of the survivors in other places had recently been stepped on and broken. Half a dozen new seedlings were found in this part of the area, a few under trees and bushes, a few in the open by a new excavation.

The fenced pasture extended only to the road, and the area east of the road had not been regularly grazed. The single plant on the trail behind the old stable site was gone, as were all but five of the twenty plants around the fallen tree. However, all the other plants present in 1947 to the east of the road seemed to be still present and healthy and a few new ones had come up here and there. The biggest outburst, including about twenty new seedlings, was on a pile of orchid peat dumped the year before near the old stable site.

Down the hill, the solitary individual in the woods near the barn was gone. The other poke plant at the corner of the cornfield was still alive and still alone.

In the river bottom, the colony at the western edge of the area seemed to be about the same size as before, although the plants were immersed in a dense mass of other herbs which had come in since 1947. The colony of a dozen plants on the south edge of the river had been carried away, along with many tons of river bank, by stream cutting. The habitat of the other river-bottom colonies remained essentially as before—open woods with some minor new cutting and filling. The previous mapping had not been sufficiently precise to permit spotting many of the former plants as individuals, but the population of mature plants as a whole had held its own and a moderate number of new seedlings were scattered through this part of the river bottom.

OTHER HABITAT RECORDS.—

The diverse habitats occupied by poke in the area of field study, including both damp river-bottom woods and well-drained open uplands, appear to be characteristic of the species over most of its range. Habitat notes in published floras and on herbarium specimens indicate that poke is able to tolerate a remarkable variety of temperature, light, and moisture conditions. Its micro-distribution appears to be limited by climatic conditions only near the northern and western margins of its range. Moreover, soil texture and acidity do not appear to be the usual limiting factors. Poke is common in clayey as well as sandy soils and tolerates a wide range of pH (Palmer and Steyermark, 1935; Deam, 1940).

The only factor common to all the reported habitats is the ever-recurring theme of disturbance of the soil and of the plant cover. A canvass of habitat notes from all available sources shows that two classes of sites are repeatedly mentioned from all parts of the range. One group of notations involves sites dis-

rupted by man's activities: old orchards, gardens, old pastures, hog-yards, neglected barn-yards, dumps, clearings, burns, and habitation sites. The other group of notations indicates no artificial disturbance but mentions places where stream erosion and deposition would be expected: ravines, river banks, low woods near creek, river-bottom woods, woods flooded in spring, alluvial woods, low ground.

It should be mentioned in passing that association with disturbed habitats is not peculiar in the genus *Phytolacca* to *P. americana*. The weedy behavior of two tropical American species of *Phytolacca* and their hybrids has already been described from Fassett's field observations in Colombia (Fassett and Sauer, 1950). The fragmentary available information indicates that most other members of the genus are characteristically weedy also.

SEED DISPERSAL AND VIABILITY.—

The association of poke and disturbed ground is most intimate and most apparent in the case of seedling plants. Old well-established poke plants can hang on for at least a few years in the face of considerable competition from other herbs; quite early in the season they are able to produce vigorous leafy shoots from their great perennial roots. Seedlings start growth later in the spring and develop relatively slowly. Where they must compete on an equal footing with other herbs, poke seedlings appear to be at an almost hopeless disadvantage. One of my experimental field plantings, given only an initial cultivation, was almost completely killed out by heavy growth of amaranths and other weeds which came up after the poke seedlings were well started.

In order to exploit the shifting and temporary spots of bare soil where they are free from choking competition, these plants require an efficient seed dispersal mechanism. Poke is poorly equipped for dispersal by wind or water. The mature fruits, with the seeds embedded in the slowly drying berry, remain firmly attached to the inflorescence even as the stalk dies. Dispersal ordinarily occurs only if the fruit is picked and transported by some animal. Birds are without doubt the usual dispersers of poke seed, as is suggested by the close association of poke with bushes and fences as well as by the sudden outbursts of isolated new colonies. It is well known that birds eat pokeberries frequently and regularly; at times the berries form one of the chief foods of the smaller migratory birds (Shultz, 1795; Grieve, 1931; Parks, letter).

The rapidity with which quantities of poke seedlings spring up in freshly disturbed sites, far from any former colonies, suggests the possibility that disturbance sets off germination of dormant seed. Poke seeds are viable for an extremely long time. Seeds buried in 1902 at depths too great for germination gave 80 to 90 per cent germination upon being unearthed in 1941 (Toole, 1946). Their long life would allow poke seeds to accumulate in the soil from occasional bird droppings over a long period of years until some disruption provided conditions suitable for germination.

Conclusion

The distribution patterns of poke suggest possible answers to the questions of what peculiar characteristics have enabled poke to exploit disturbed places, what habitats it occupied in pre-human times, and how the species has been affected by its life with man.

In spite of being weak in competition and poorly equipped to spread by methodical progressive advance, this plant has some special characteristics which have made it a successful weed. One of these is its relatively broad tolerance of light, microclimate, and soil conditions. Equally important are the production of berries attractive to birds and the long life of the seeds. Wide bird dispersal and high seed viability, even after many years of dormancy, give poke a head start over its competitors in the race to colonize isolated spots of newly opened ground. Given sufficient head start, mature poke plants, with their great perennial roots, can survive among more competitive but later arriving herbs for enough years to produce their contribution of seed.

The micro-distribution patterns of poke provide what seems to be a significant clue to the ancient habitat of the plant: Everywhere, even in the heart of its native range, poke is bound to disturbed sites and nowhere does it seem to belong to a stable plant association. Poke seedlings appear to be able to establish themselves only where some external factor has intervened to obliterate the potential competitors and open up a patch of raw soil. Before the coming of man, poke could have found a niche in habitats disrupted by natural agencies. Its stronghold may have been in open stream-bank woods, where new ground was constantly opened by cutting and filling. Poke undoubtedly colonized other natural scars in the mantle of vegetation—gullies, landslides, burns, blowdowns—but away from the constant intervention of the stream such colonies could ordinarily persist only briefly until they were overwhelmed by the slow advance of more aggressive vegetation.

With the invasion of North America by man, the area of ground bared to poke colonization must have increased, slightly at first with the earliest primitives, considerably more as Indian agriculture spread, and enormously since the European settlement. In its native area, the general range of the species appears to be climatically controlled and may have been changed very little by human activity. Certainly, there is no evidence of migration by the plant in this region in historic times. Within the old limits, poke colonies must have multiplied and spread in artificial habitats until today the colonies occupying naturally disrupted sites form a minor and easily overlooked part of the greatly expanded population.

Outside its native area, the general range of poke has been greatly extended by man in the last few centuries. Because of its useful properties the plant was deliberately introduced into other continents, where it has naturalized itself as a minor weed.

With the great expansion of the poke population in artificial habitats, new strains especially adapted to the new conditions might have been expected to evolve. So far as I can judge, there is no evidence that such evolution has taken place. The poke of natural river-banks and forest blowdowns and the weed of settlement margins and abandoned fields are morphologically indistinguishable. Poke's success as a weed of the cultural landscape appears to be based, not on evolution during human times, but on its previous adaptation as a pioneer species of naturally disturbed places. The story of poke is thus of a different nature than the stories of those weeds, including many tropical *Phytolacca* populations, which are the product of recent hybridization and selective modification in artificial habitats.

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THE GAMETOPHYTE OF *CARDIOCARPUS SPINATUS* GRAHAM

HENRY N. ANDREWS* AND CHARLES J. FELIX**

Among the more interesting fossils that we have encountered in coal balls from West Mineral, Kansas (Andrews, 1951), is a seed, presumably of cordaitean affinities, with a rather well-preserved gametophyte. It seems to be referable to *Cardiocarpus spinatus* Graham (1935, p. 165), although it presents much additional information relative to the structure of the integument and gametophyte. The final selection of a name for the seed presented a considerable problem, and because of some general principles of seed nomenclature, it seems desirable to discuss in detail certain historical aspects of the case.

In 1828 Brongniart recorded (Prodrome, p. 87) the genus *Cardiocarpon* and listed five species. The only description given is as follows: "Fruits comprimés, lenticulaires, cordiformes ou réniformes, terminés par une pointe peu aiguë." Brongniart did not deal with the genus in his monumental 'Histoire des Végétaux Fossiles' (1828–1838) and seems to have had little or nothing to do with it until 1881 when his work on the silicified seeds appeared. Various other authors, however, have described species of *Cardiocarpon,* some soon after 1828, and the variation in form illustrated composes a rather incredible array of what are apparently many generic entities. A few examples will suffice to reveal the lack of recognition of any clearly defined generic boundary. Mantell (1844, p. 153, fig. 34-1) figures under the name *Cardiocarpon acutum* a round strongly winged seed; the wing apparently entirely encloses the body of the seed. Lindley and Hutton (1831–37, pl. 76) illustrate wingless seed casts under the same name. Dawson (1878, fig. 194b) pictures a seed with a strongly attenuate apex under the same binomial; and Williamson (1877, pl. 15, fig. 122) figures a bicornute seed under the name "*Cardiocarpon acutum* of Lindley and Hutton," although the binomial was originally Brongniart's (1828, p. 87). In 1853 Newberry described *Cardiocarpon samaraeforme* as a nearly round seed with two large and distinct wings; while Dawson's figures show *Cardiocarpon cornutum* as an elongate winged seed with cornute apex.

The above is a very brief introduction into the almost endless range of form of the seeds gathered together under this generic designation. More fuel was added to the flame of confusion when in 1862 Geinitz created the genus *Cordaicarpon,* the generic description differing in no significant way from that of Brongniart's for *Cardiocarpon.* Geinitz (1855) described *Carpolithes cordai* (which he believed to be the seed of *Cordaites principalis* (Germar) Geinitz) and designated it (in 1862) as the type species of his new genus. In referring to his illustrations (Geinitz, 1855, pl. 21, figs. 7–16), we find it impossible to recognize any characters which set his genus apart from Brongniart's or in any way justify a distinct genus. To further confound later workers these generic names, originally given as

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Cardiocarpon Brongniart and *Cordaicarpon* Geinitz, have since been variously cited as *Cardiocarpus* or *Cardiocarpum* and *Cordaicarpus* respectively.

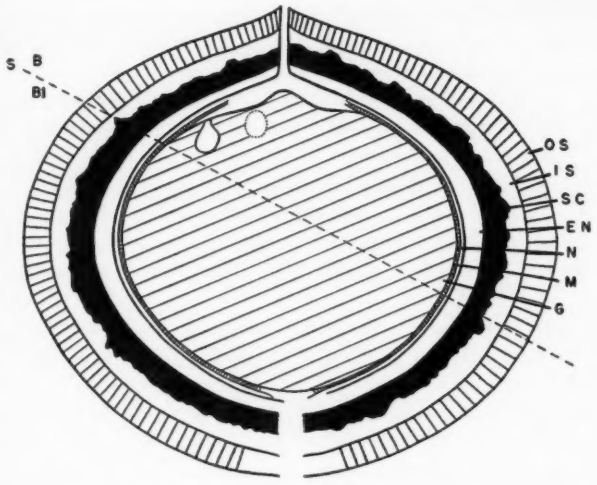
The next, and indeed significant, phase in the history of these fossils was Brongniart's description in 1881 of petrified seeds which he assigned to *Cardiocarpus*, thus emending the original generic concept (of *Cardiocarpon*) to include structurally preserved remains.

In an effort to alleviate the chaotic state in which these two genera existed, Seward (1917, p. 338) proposed that *Cardiocarpus* be restricted "to petrified seeds exhibiting the characters described by Brongniart" in 1881, and that *Cordaicarpus* should serve "for platyspermic seeds, preserved as casts or impressions, having a comparatively narrow border enclosing an ovate or cordate-ovate nucule; the base is either rounded or cordate" (p. 354). Since the spellings employed by Seward have been used by most workers for several decades, it seems to be most expedient to continue them.

Seward's usage of these two generic names was intended to be useful, and his desire to create a workable basis for dealing with such fossils is commendable. However, a system of classification based on mode of preservation is apt to run into difficulties sooner or later, the introduction of better techniques and discovery of intermediate types of preservation presenting notable obstacles to such a system. Thus, a complicating but interesting link was presented by Miss Reed (1946) when she described certain compression fossils preserved in shaly limestone from Iowa in which a considerable amount of cellular detail is recognizable. She has provisionally referred these specimens to "*Cardiocarpon affinis* Lesquereux." Further consideration of this fossil will be taken up below.

We may next consider *Cardiocarpus spinatus*, a seed described by Graham (1935) from an Illinois coal ball. Judging from the description, his specimens were very poorly preserved; his accompanying figures show no cellular detail. Apparently, the distinctive feature of the seed lies in the spiny nature of the sclerotesta. Darrah (1940) refers to this fossil under the name *Cordaicarpus spinatus*. It is not clear why he uses Geinitz' genus *Cordaicarpus* which was based on impression specimens and which Seward (1917, p. 354) proposed to continue in use as a genus in which casts and impressions would be relegated. It seems evident that Darrah had better-preserved material available, although his description is brief and the single illustration (fig. 22) given is at too low a magnification to be informative.

We now return to the fossils in our own collections which we believe are referable to *Cardiocarpus spinatus* Graham and which contribute further to our knowledge of the structure of this seed. This description is based on two coal-ball specimens from the Fleming coal which occurs in the upper part of the Cherokee shale, Des Moines series, middle Pennsylvanian, about four miles south of West Mineral, Kansas. One specimen (No. 783) contains a well-preserved gametophyte and the other (No. 721) is significant for the preservation of the integument.

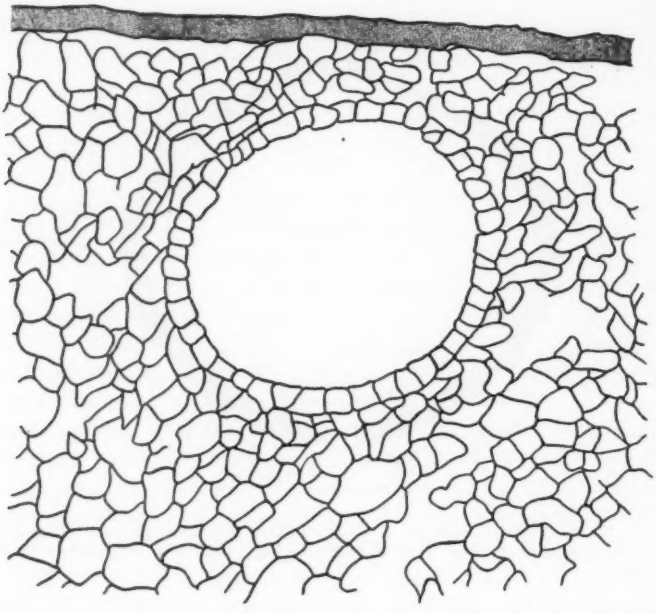


Text-fig. 1. *Cardiocarpus spinatus*, a diagrammatic longitudinal section: G, gametophyte; M, megaspore membrane; N, nucellus; EN, endotesta; SC, sclerotesta; IS, inner sarcotesta; OS, outer sarcotesta.

The initial saw-cut of No. 783 which exposed the seed was made at an angle of approximately 30° to transverse as is indicated by the dotted line "S" in text-fig. 1. Two series of peels were started from the respective surfaces, one going toward either end, although at the initiation of these series it was not possible to be sure of the exact orientation of the seed. Thus a series of peels was started in the "B" direction which are labelled 783-B-S1, etc., and a series in the B1 direction which are labelled 783-B1-S1, etc. When at peel B-S 4 a structure appeared which gave evidence of being an archegonium and was confirmed in the following peels, it was evident that the "A" direction was the micropylar one. A total of 48 peels was made in this direction although the apex of the gametophyte was passed at B-S36. A series of 30 peels was made toward the chalazal end labelled B1-S1 to B1-S30, at which point a tangential series was started parallel to the broad lateral face, these being labelled B1-S'1, etc.

It may be noted that all measurements given below are corrected to account for the 30° divergence of these peels from the true transverse.

In a median section the seed containing the gametophyte measures 11.0 × 7.5 mm. The following tissues may be noted (text-fig. 1; pls. 13 and 14, figs. 1, 4, 5), starting from the inside: *G*, gametophyte; *M*, megaspore membrane; *N*, nucellus; and the integument consisting of: *EN*, endotesta, the cellular structure of which is very poorly preserved; *SC*, sclerotesta, the conspicuous portion of the integument characterized by its spiny outgrowths (the walls of this tissue are



Text-fig. 2. *Cardiocarpus spinatus.* Enlarged view of an archegonium as shown in the upper part of fig. 2, pl. 13. A few of the jacket cells have been restored. Stipple band indicates megaspore membrane.

somewhat thicker than those of the cells composing the sarcotesta outside but not as markedly so as might be assumed from a casual observation[1]); and finally the thick fleshy sarcotesta which is composed of two strikingly different tissues (figs. 4, 5)—an inner region, *IS*, which extends hardly beyond the outer limits of the spines of the sclerotesta, and an outer region, OS, which is distinguished by much larger cells. In fig. 1 only the inner sarcotesta is preserved.

While studying a coal ball (No. 721) containing a medullosan stem from the same locality we encountered another seed in which an undoubted complete sequence could be observed from sclerotesta to epidermis (figs. 4–6). Here it may be noted that the cells of the inner sarcotesta are small (averaging about 55 μ in diameter) when compared with those of the outer sarcotesta which are about three times as large. The latter are delimited by a clearly defined epidermis (fig. 6). The extreme rarity of this outermost tissue in the fossils is understandable in view of the relatively large size of the cells and their thin walls; judging from its thickness (about 1 mm.) in the one specimen we have in which it is well preserved, this would give a revised statement of the diameter of the seed as 13.0 $\times$ 9.5 mm. instead of the 11.0 $\times$ 7.5 cited above.

[1] In most specimens it is only the sclerotesta that is preserved. We are inclined to believe that the durability of this tissue is to be attributed in part to the preservative quality of the cell contents as well as to the slightly thicker walls.

The gametophyte.—

As far as its gross cellular organization is concerned, the gametophyte is almost entirely intact. The intercellular substance appears to have decayed so that the cells have the appearance of being loosely held together although there is no appreciable distortion. The cell walls are clearly defined when observed by reflected light, yet the little organic matter remaining renders the walls so very light in color that it is not possible to obtain satisfactory photographs.

The gametophyte measures approximately 8.7 × 4.0 mm. in transverse section and about 7.5 mm. in length. It is ovoid except for the apex which appears to display the usual "tent pole" form. The latter is evident in oblique section in peel B-S35.

Two archegonia are present in the gametophyte. As indicated above, one begins to appear in B-S4, attains its maximum diameter of 0.5 mm. at about B-S10, and at S18 it appears to open out. The second archegonium appears between peels B-S23 and B-S32. Both of the archegonia are on one side of the seed, one being somewhat above the other. Little else can be said other than the fact that these organs are delimited from the rest of the gametophyte by a jacket of conspicuously smaller cells (text-fig. 2).

Other Carboniferous female gametophytes.—

Several examples of well-preserved gametophytes are now known from the Pennsylvanian, the lycopods being most abundantly represented. Scott (1901) described the gametophyte of *Lepidocarpon lomaxi* and *L. wildianum*, and more recently Andrews and Pannell (1942) described the gametophyte of the American *L. magnificum*. Schopf (1941) has given a good account of the gametophyte of *Mazocarpon oedipternum*, and Darrah (1938) has recorded a *Selaginella* gametophyte with exceptionally striking nuclear details. Other fossil lycopod gametophytes have been described by McLean (1912) and Gordon (1910).

Referring to the Carboniferous seed plants proper, Brongniart (1881) has figured gametophytic tissue in several seeds from St. Etienne in the genera *Cardiocarpus*, *Leptocaryon*, *Rhabdocarpus*, *Taxospermum*, and *Stephanospermum*. Although his beautifully executed illustrations show what is apparently the gametophyte, and in several instances the position of the archegonia and egg cells, clearly defined cellular details are not given. More recently Long (1944) has described the gametophyte of *Lagenostoma ovoides* in an excellent state of preservation.

Taxonomic considerations.—

There seems to be no doubt that the specimens of *Cardiocarpus spinatus*, originally described by Graham (1935) and later by Darrah (1940), are specifically identical with those described here. In view of the fact that our specimens add appreciably to our knowledge of the seed an emended description is given below.

It seems important also to comment on the semi-petrified seeds described by Miss Reed (1946) as *Cardiocarpon affinis* Lesquereux. Her contribution is interesting and significant, particularly from the standpoint of correlating impression or compression fossils with typically petrified ones, and it clearly points out the difficulty of retaining without revision Seward's classification of these seeds. There is, moreover, little doubt in our minds that the coal-ball seeds of *Cardiocarpus spinatus* are specifically identical with Reed's specimens. The problem therefore lies in specifically correlating all of these with Lesquereux's specimens, first described by him as *Cardiocarpon affine* (1860, p. 311) and later as *Cardiocarpus affinis* (1880, p. 564), the latter spelling being the acceptable one in our opinion. In an attempt to settle the problem we have examined Lesquereux's type specimen which is preserved as No. 8038, Paleobotanical Collections, Botanical Museum, Harvard University, and we have illustrated it in fig. 3. It is hardly more than an impression, there being but little organic matter present. It conforms very closely in size and shape with our coal ball specimens, but it displays no evidence of the spiny sclerotesta which is the most distinctive feature of the seed. It is of course possible that decay took place before a durable impression of this tissue could be left in the matrix. However, lacking this evidence we do not feel that there is adequate justification for including any of the other specimens mentioned above under Lesquereux's binomial.

Cardiocarpus spinatus Graham, emend Andrews & Felix.—

Platyspermic seeds with integument consisting of endotesta, strongly spinose sclerotesta, and sarcotesta, the latter being composed of two distinct zones; cells of the outer sarcotesta about three times as large (diameter) as those of the inner sarcotesta; seeds approximately 13.0 × 9.5 mm. in diameter, and containing a gametophyte bearing two archegonia.

Origin of specimens.—

Graham, 1935: McLeansboro group, Pennsylvanian; Calhoun coal mine, Richland County, Illinois.

Darrah, 1940: Des Moines series, Pennsylvanian; Shuler and Urbandale coal mines, Waukee, Iowa.

Andrews and Felix: Cherokee shale, Des Moines series, Pennsylvanian; four miles south of West Mineral, Kansas.

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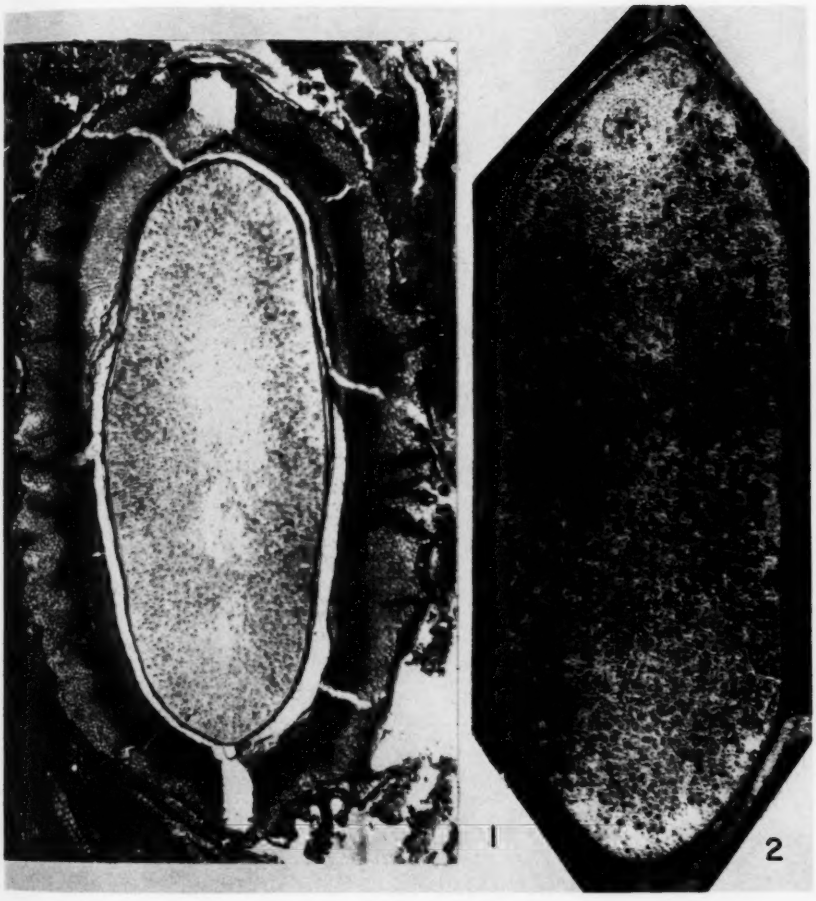
Explanation of Plate

PLATE 13

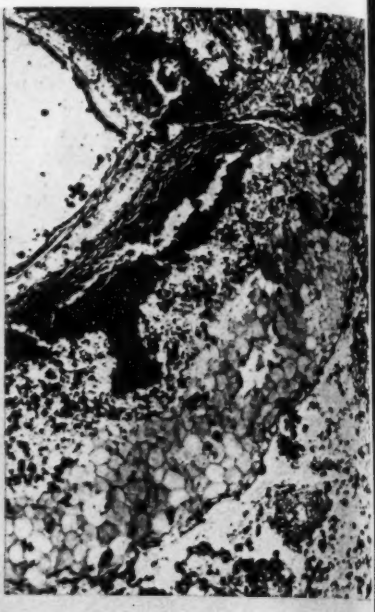
Cardiocarpus spinatus Graham

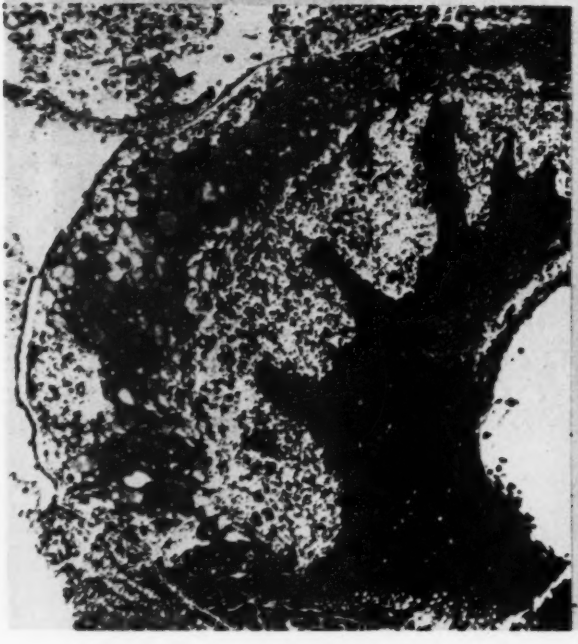
Fig. 1. A nearly transverse section through a seed containing a gametophyte. Actual plane of the section is indicated by dotted line "S" in text-fig. 1. The outer sarcotesta is not preserved in this specimen. Peel 783-B1-S18.

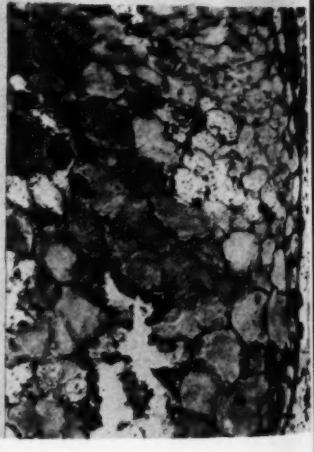
Fig. 2. Enlarged view of the gametophyte showing an archegonium in the upper part of the figure. Peel 783-B-S13.



ANDREWS & FELIX—*CARDIOCARPUS SPINATUS*







ANDREWS & FELIX—*CARDIOCARPUS SPINATUS*

Explanation of Plate

PLATE 14

Fig. 3. *Cardiocarpus affinis* Lesquereux. Photograph of the type specimen, No. 8038, Paleobotanical collections, Botanical Museum, Harvard University. × 12.

Cardiocarpus spinatus Graham

Figs. 4, 5. Transverse sections of the seed showing well-preserved outer sarcotesta. Slide No. 1967. × 19.

Fig. 6. The outer sarcotesta and epidermis enlarged. Slide No. 1967. × 54.

FACTORS AFFECTING THE MORPHOLOGY OF *CANDIDA ALBICANS**

DAN OTHO McCLARY**

INTRODUCTION

Most morphological studies on the yeast-like fungi have been conducted on natural substances—malt extract, corn meal, and various vegetable decoctions such as potato and carrot, as broth or solidified with agar. Since growth on these chemically unknown substances yields a great variety of morphologically different forms, there is much controversy as to their true morphologies and the causes for their variations. For this particular group of fungi and perhaps for others which are somewhat more stable morphologically, more precise physiological information seems to be needed than can be obtained on natural media in order to arrive at definite conclusions concerning morphological variation. It is the purpose of this study to define the morphology of a well-known species grown in media of known chemical composition under carefully controlled physical condition, in the belief that much of the existing confusion in the taxonomy of this group of fungi can be eliminated by use of such an approach. *Candida albicans* was chosen because of its extreme variations in form, and because of the extensive studies which have been made upon it.

I am indebted to Dr. Carroll W. Dodge for suggesting this problem and for his generous help throughout the course of the study.

Classification.—Although the yeast-like organism described by Robin in 1847 as the cause of the disease known in modern literature as thrush, muguet, sapinho, and Soor has been known for over a hundred years, there is apparently little agreement among mycologists as to its taxonomic position or even its name. Robin (1853) first named the organism *Oidium albicans*. Quinquaud (1868), realizing that the organism did not belong in *Oidium*, placed it in his new genus, *Syringospora*, naming it *Syringospora Robinii*. He not only described the characteristic clusters of blastospores, but he also presented drawings definite enough for one to be reasonably certain that he referred to the organism now known as *Candida albicans* (Dodge, 1935; Skinner, 1947). In 1877 Grawitz called attention to the differences between the yeast form and the mycelial form. He also described chlamydospores and even discussed the action of the media on morphology; but it is thought that he may have been working with mixed cultures because of his crude culture techniques. He believed this organism to be the same as *Mycoderma vini*. Reess, in 1877, showed that the organism was distinct from *Mycoderma vini* and called it *Saccharomyces albicans*. Plaut, in 1885, was the first to apply modern cultural technique. He identified the mycelial form with *Monilia candida* Bonorden on decaying wood. Stumpf, in 1885, concluded that he had two organisms, one

* An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the degree of Doctor of Philosophy.

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filamentous and one yeast, both liquefying gelatin. In 1885 Baginsky studied the organism on various media, and Klemperer produced experimental mycosis from intravenous injections.

Audrey (1887) first proved the connection between yeast and mycelial forms, showing that yeast cells were more common on solid media, filaments in liquid. Roux and Linossier (1890) studied the physiology in considerable detail, giving extensive notes on carbon and nitrogen metabolism without definitely describing the biochemical reactions. They describe their organism as producing white, elevated, creamy colonies, with surface slightly furrowed on cooked carrot. At first the yeast cells predominate, then there are some filaments for a short period, and finally yeast cells again. On liquid media the filamentous forms predominate except in malt extract. On most fruits (except melon) and on peptone gelatin, the yeast form is abundant. On sucrose gelatin both forms are found. No ascospores were observed, but chlamydospores were not uncommon on most media used.

In 1923 Berkhout introduced the new genus, *Candida*, and designated *Monilia candida* Bonorden 1851 as the type species based on a culture isolated by Kloecker under the name *Monilia candida* but "evidently not that species" (Dodge, 1935). To avoid the use of a repeating binomial, Berkhout changed the name to *Candida vulgaris*.

In 1934 Diddens and Lodder adopted Berkhout's genus, *Candida*, but they designated another species, *Candida albicans*, as the type. This name has persisted in spite of the objections of most of the other well-known mycologists including Dodge (1935), Conant (1940), Mackinnon and Artagaveytia-Allende (1945), and Skinner (1947). All agree that it should be *Syringospora* Quinquad 1868 by right of priority. This organism appears in the literature under a number of other names, but most of the important work concerning it may be found under *Monilia*, *Syringospora*, and *Candida*. The organism used in this study was received from the American Type Culture Collection as *Candida albicans*, and that name will be used in this paper.

General Morphology.—There are many morphological descriptions of this highly pleomorphic organism in the literature (Quinquaud, 1868; Grawitz, 1877; Audrey, 1887; and many others). The close correlation between the physiology and morphology of this organism is generally recognized, so that culture conditions and the medium used are always given with the morphological description. Skinner's description (1947) is a generally accepted one:

> Except for the chlamydospores there is little other morphological detail that will set a strain of *C. albicans* apart from the other species. Freshly isolated cultures show little tendency to formation of true or pseudomycelium unless grown in starvation media below the surface, as in scratch corn meal agar plates or potato infusion broth, or in sugar-free beef peptone gelatin stabs. Grown on ordinary Sabouraud agar the cells are almost exclusively of the budding yeast type. Strands of mycelium may penetrate into the substrate after prolonged incubation, but they are much more numerous and appear more promptly along the scratch in corn meal agar. Blastospores are invariably produced from the strands, but the arrangement of blastospores varies so much between isolates that discussion of this

has little value in a review of this sort. They tend to occur in ball-like clusters in fresh isolates, but not to the extent that they do in *Candida albicans* var. *stellatoidea*.

Morphological variation.—Morphological variations described in the literature are of two distinct types:

1. Irreversible changes called "degeneration" (a seemingly gradual change) and "dissociation" (a sudden but irreversible change) involving a mutation.
2. Reversible changes depending entirely on environmental conditions.

Irreversible changes.—This type of variation has been studied by Negroni (1935), Mackinnon (1940), Mickle and Jones (1939), Cavallero (1939), Martin and Jones (1940), and Conant (1940). Mackinnon (1940) described "membranous variants" and "lethal variants." In the "membranous variants" the blastospores become elongated into filaments, causing a characteristic wrinkled, or in more advanced variants, a spiky hard colony surrounded by a filamentous halo. In liquid medium this variant produces a mucous veil and the virulence diminishes. The biochemical properties do not suffer qualitative changes. The "lethal variation" is characterized by a lower rate of growth, a great diminution or total loss of virulence, and by increasing difficulty to produce mycelial growth. These variations may occur spontaneously as described by Mackinnon, or they may be induced by toxic substances such as immune serum (Negroni, 1935) or by lithium chloride and immune serum (Mickle and Jones, 1939).

Although the existence of these "dissociations" are accepted by most mycologists, Langeron and Guerra (1939) concluded, after their investigations of these "irreversible variations" made over a period of some ten years, that the S (smooth phase) is the normal one and the R (rough phase) develops as a result of various factors, chief of which are the reaction (pH) of the medium and "elongation factors" (presence of carbon dioxide, nutrients of a high molecular weight, and nitrates). These variations were reversed when the organism was transferred to fresh media. They did not find irreversible variations as reported by Mackinnon and others.

Reversible changes.—It is with this type of variation which occurs promptly when the organism is transferred from one set of culture conditions to another that this study is primarily concerned. In 1930 Talice published perhaps the most complete study and review of the factors influencing the reversible changes in this organism. He determined that production of filaments depends upon partial anaerobiosis, weak concentrations of nutrients in the culture medium, the strain of the organism used, the treatment it has undergone, and the age of the culture. He believed that the filamentous form is always the young form; the yeast form is the old form.

In 1938 Langeron and Guerra found the formation of filaments to be stimulated by prolonged culture in the laboratory, presence of high concentrations of carbon dioxide, and changes in the constituents of the medium during the course of growth, particularly the change in pH. Morquer and Nystérakis (1948) reported that certain concentrations of heteroaxine (beta-indole-acetic acid) stimulate filament formation.

Nickerson and Jillson (1948) found that a metabolic product of *Trichophyton rubrum* would inhibit the filamentous phase of *Candida albicans* but had no effect on the yeast phase. They considered that a separate enzyme system controlled each of these two phases and that the morphology of any given culture depended upon a stimulation or suppression of one or the other of these two systems which are supposedly competing for the same substrate. Nickerson (1950) again attributed the morphology of the yeast-like fungi to a delicate balance between growth and cell division. If the balance were upset in such a manner as to permit only growth to occur, elongated cells without cross walls would be formed. Cell division is, according to him, associated with the maintenance of intracellular sulfhydril (-SH) groupings. In slide cultures of *Candida albicans* grown on a synthetic medium consisting of glucose, ammonium sulfate, inorganic salts, and biotin, Nickerson found only the yeast form. When commercial or purified starch was substituted for the glucose, abundant filamentation and chlamydospores were produced. By adding cysteine to the medium, filamentation and chlamydospore formation were prevented and only yeast cells were formed. He concluded there must be a certain amount of a readily assimilable carbohydrate such as glucose in order to maintain the high oxidation-reduction potential essential for the intracellular -SH groupings required for proper cell division.

In general, one must conclude from the findings in the literature that where conditions are favorable for rapid multiplication, as with easily assimilable carbohydrates and with abundant aeration, the unicellular yeast forms predominate. Reduced oxygen tension, starvation media, liquid media in general, high pH, high temperature, or practically any condition or set of conditions which inhibits growth but does not stop it entirely, tend to produce the mycelial growth of *Candida albicans*.

General physiological characteristics.—In practically every taxonomic work dealing with this organism, its ability to produce acid and gas from various carbohydrates has been used. Most workers agree that the organism produces both acid and gas from glucose, fructose, mannose, and maltose; acid and sometimes gas from galactose; and acid but never gas from sucrose. Kluyver and Custers (1940) and van Niel and Cohen (1942) have published papers concerning the biochemistry of carbohydrate fermentation of the yeast-like organisms. According to van Niel and Cohen, there is no essential difference between the fermentation of glucose and sucrose by *Candida albicans* except that it occurs at a considerably faster rate in glucose.

In addition to carbohydrate fermentation tests, most authors also include a study of various nitrogen compounds as possible sources of assimilable nitrogen for the organism. Most of this work has generally been on synthetic media consisting of a sugar for a carbon source and inorganic salts (auxanograph). Wickerham (1946) showed that certain nitrogenous compounds which have been reported as unassimilable are readily used if the proper growth factor or

factors is present in sufficient quantity in the auxanograph medium. Burkholder (1943), using chemically defined media, found that biotin is required for the growth of *Candida albicans* and that thiamin is stimulating. The work of Morquer and Nystérakis and of Nickerson and his co-workers was done largely upon chemically defined media. However, except for the substitution of starch for sugar by Nickerson in one of his media, their work consisted of studying the various substances which are supposed to have certain physiological effects on cell elongation or cell division. There has apparently been no attempt to determine the effects of altering various other essential components of the medium.

Methods

The organism used for the greater part of this study was obtained from the American Type Culture Collection as *Candida albicans 2091*. Five other cultures were sent by Dr. J. E. Mackinnon at the request of Dr. Carroll W. Dodge. These organisms were maintained on media consisting of: glucose, 1 per cent; Difco yeast extract, .5 per cent; and agar, 2 per cent.

Culture media.—The medium used, essentially that described by Olson and Johnson (1949) and hence to be referred to as "basal medium," is as follows:

Sucrose	10.0 gm.	Calcium pantothenate	1.0 mg.
Potassium chloride	1.0 gm.	Inositol	5.0 mg.
KH_2PO_4	0.2 gm.	Thiamine-HCl	200.0 microgm.
$MgSO_4.7\ H_2O$	10.0 mg.	Pyridoxine	200.0 microgm.
Amm. citrate (dibasic)	6.0 gm.	Zinc sulfate	400.0 microgm.
Calcium chloride	50.0 mg.	Ferric ammonium	250.0 microgm.
L-asparagine	2.0 gm.	Copper sulfate	25.0 microgm.
Biotin	2.5 microgm.	Bacto agar	20.0 gm.
Riboflavin	75.0 microgm.	Distilled water to	1000.0 ml.

To facilitate the preparation of the several media required, stock solutions of vitamins and trace elements were prepared, preserved with toluene, and stored in the refrigerator.

In addition to variations of the above medium, various natural media with modifications as indicated were used. These include Bacto yeast extract, Bacto peptone, Bacto malt extract, corn steepwater, Bacto beef, Bacto corn-meal agar, and potato and carrot decoctions. Various sugars (C.P.) and other chemicals were used as indicated.

Although most mycologists insist that morphological studies should be made *in situ* as cover-glass or slide cultures or that the cultures be examined directly on the petri plate (Skinner, 1947), this method has been used to only a limited extent in this study. Almost all this work was carried out on agar slants or broth cultures in test-tubes. This method was believed necessary for at least four reasons: (1) With the many hundreds of cultures used in such a study, so much time would be consumed in making microcultures that they could not very well be continuously observed. (2) Slide and petri-dish cultures, when subjected to prolonged examination, become much more easily contaminated. (3) It was found that, probably due to the rapid exhaustion of nutrient material, slide cultures did

not undergo all the changes which were observed on slants. (4) A macroscopic as well as a microscopic examination was desired for each culture. Petri-dish and cover-glass cultures were, therefore, used only to verify observations made on slant cultures.

Slides for microscopic examination were made by transferring a bit of material from the slant to a slide upon which had been previously placed a drop of water or staining solution. A nichrome wire hook bent at a right angle about ⅜ inch from the end was used rather than a loop because the cultures were, under certain conditions, so tough that they resisted any amount of pressure that could be applied to them with a loop. Acetocarmine was found to give excellent results, the organism staining a bright red against a relatively colorless background. Since this stain evaporates quite rapidly, it was necessary to seal the preparation soon after the cover slip was in place. Turtox slide-ringing cement obtained from the General Biological Supply House, Inc., Chicago, Illinois, was found to be very satisfactory for sealing.

Verification of the Species

For morphological verification, agar slants of basal medium, .5 per cent yeast extract, and .5 per cent malt extract were inoculated with *C. albicans* A.T.C.C. 2091 and incubated at room temperature (25–30° C.).

For biochemical verification two types of media were used: (1) .3 per cent peptone, 10 per cent gelatine in distilled water for gelatine liquefaction tests; and (2) a series of nine carbohydrate media consisting of .3 per cent peptone with brom thymol blue indicator, and sugars as follows: (1) glucose, (2) fructose, (3) mannose, (4) galactose, (5) maltose, (6) sucrose, (7) lactose, (8) trehalose, (9) no addition.

The gelatine was dispensed in "18 × 150 mm." test-tubes; the carbohydrate media in large Smith fermentation tubes, and all were autoclaved at 12 pounds pressure for 15 minutes. After cooling, tubes of gelatine were inoculated in duplicate, using the stab method, and incubated at 25° C. The fermentation tubes were inoculated in duplicate with a small amount of culture taken with a hook from an agar slant and were incubated at 37° C. for five days.

In addition to the above fermentation tests, Durham fermentation tubes were prepared, using "23 × 185 mm." test-tube with a "10 × 75 mm." test-tube for a gas vial. A fermentation medium consisting of 3 per cent peptone in distilled water with brom thymol blue indicator was divided into three parts and 5 per cent quantities of the following sugars were used in each respectively: (1) glucose, (2) sucrose, and (3) galactose. The fermentation tubes were inoculated very heavily—each one being inoculated with approximately all of a slant culture which had been grown previously, using a corresponding sugar as a carbon source. It was hoped that the great number of cells initially present would provide anaerobic conditions.

Results.—Microscopic examinations of slides prepared from bits of the cultures obtained from near the center of the slants revealed, in all cases, a complex mixture of filaments with verticels of blastospores and budding yeast cells. After several days, there were also observed numerous thick-walled, round chlamydospores appearing terminally on thick filaments and free in the medium. The results of the fermentation tests with a light and a heavy inoculation used in this study are given in tables I and II, respectively.

TABLE 1

ACID AND GAS PRODUCTION BY *CANDIDA ALBICANS* A.T.C.C. 2091 IN PEPTONE CARBOHYDRATE MEDIA. LIGHT INOCULUM.

Carbohydrate	Reaction	Gas
Glucose	Acid	+*
Fructose	Acid	+
Mannose	Acid	+
Galactose	Acid	—
Maltose	Acid	+
Sucrose	Acid	—
Lactose	Strongly alkaline	—
Trehalose	Acid	—
None	Strongly alkaline	—

*+, gas produced; —, no gas produced.

TABLE II

ACID AND GAS PRODUCTION BY *CANDIDA ALBICANS* A.T.C.C. 2091 IN PEPTONE CARBOHYDRATE MEDIA. VERY HEAVY INOCULUM.

Carbohydrate	Reaction	Gas
Glucose	Acid	+*
Sucrose	Acid	—
Galactose	Acid	+

*+, gas produced; —, no gas produced.

It is noted (Table I) that glucose, fructose, mannose, and maltose are readily fermented with acid and gas. It is also observed that, although galactose, sucrose, and trehalose are utilized, producing an acid and a rich growth, gas is produced in galactose (Table II) only under conditions unsuitable for further growth and in sucrose not at all. These observations will be again referred to in the discussion of nutrition and its relation to morphology. Finally, this organism brought about a complete liquefaction of the nutrient gelatin, a characteristic included by most taxonomists.

These results are thus in agreement with the taxonomic requirements presented in the literature and reviewed by Skinner (1947). According to Skinner, the most obvious morphological characteristic of this species is the production of round (or nearly so) heavy-walled terminal cells called chlamydospores. Strands of mycelium may or may not develop from which blastospores or buds are invariably produced. It is also generally agreed among mycologists that *Candida albicans* is the only species of the yeast-like fungi which ferments (produces gas) glucose, galactose, and maltose, but not sucrose and lactose.

Since all of the above characteristics are possessed by *Candida albicans* A.T.C.C. 2091, it is concluded that it is as typical a culture as can be obtained and is a suitable one for this type of study.

Influence of Hydrogen Ion Concentration

In this experiment, the basal medium was adjusted in series at pH 3 through 9 by means of a Beckman pH meter with approximately 5 per cent HCl and 5 per cent NaOH. The media were then dispensed in test-tubes suitable for slants and were autoclaved at 12–15 pounds pressure for 12 minutes.[1] In addition to this medium, Bacto yeast extract agar, corn steepwater agar, and Bacto malt-extract agar were prepared as above at pH values of 5 and 8. All media except that adjusted to pH 3 were inoculated as slants. The medium of pH 3 would not solidify after autoclaving and was inoculated by the stab method. These cultures were incubated at 24° C. for two days.

Results.—In general, there was little difference in growth of the organism in the ranges of pH 4 through 7. Growth was poor at pH 3 and 8, and no growth was noticeable on the pH 9 culture for several days. When growth did occur on this medium, it began as a little colony at the very thin part of the slant and gradually spread down over the thicker portion. When this colony was used to inoculate tubes of the same medium, growth occurred promptly.

On the basal medium at extreme pH ranges (3, 8, and 9), microscopic examination revealed a preponderance of yeast-like cells and large, spherical, thick-walled chlamydospores. The filaments that were present were of irregular shape and had a swollen appearance (pl. 15, fig. 1). The most filamentous growth occurred on the basal medium at pH 5. At ranges of pH 4 and 6, the filaments were not so regularly thread-like as those grown at pH 5, but they, like it, did not develop chlamydospores within 2 days. Although the culture grown at pH 7 was quite filamentous, it consisted of more yeast-like cells and irregular filaments than did those grown at a slightly lower pH. Chlamydospores were also numerous. Cultures on malt extract at corresponding pH ranges had very much the same morphology as those grown on basal medium. Yeast extract and corn steepwater produced a preponderance of yeast cells under all conditions.

[1] The effect of autoclaving was determined on these media and some change was observed. These changes were never over .5 of a pH unit, however, and always occurred in the direction of neutrality.

Influence of Nutrients

In order to determine the basic nutritional requirements of this organism, a series of media was prepared with each medium lacking a different ingredient of the complete basal medium. These media were prepared as slants, and each was inoculated from a stock culture maintained on glucose peptone agar. Slants of the complete basal medium were inoculated for controls. All were incubated at 24° C.

After one day's growth the slants were examined macroscopically; then bits of material taken from them with a nichrome wire hook were mounted on slides, stained, and examined microscopically. Examinations were made after two days, three days, and longer periods to determine the effect of prolonged incubation.

Results.—Macroscopic examination of the day-old cultures revealed considerable difference, not only in the amounts of growth on the various media but also in their gross morphologies. Poor, though distinct, growth was observed on media which were lacking in all vitamins, biotin alone, phosphorus, potassium, and sugar. All the other media except that lacking calcium pantothenate, which was so little different as to be doubtful, gave almost identically luxuriant growth.

The gross morphologies of the cultures resulting on these media were quite as distinctly different as the growth quantities. There was little difference in the growth resulting from lack of sugar, biotin or all vitamins, and phosphate, each being almost pure white, very soft, and creamy. The growth resulting on a potassium-deficient medium, though not so distinctly differing from the above in young culture, became rather dry and granular with a yellow-green color.[2] Samples of each of the above cultures could be very easily removed with a wire loop. The growth resulting on the rest of the media was a pale olive-buff or nearly white with a velvety appearance. These cultures were found to consist of a distinct, tough membranous mat covering the surface of the agar. It was necessary to use a wire hook to tear pieces of this membrane from the slant. With little difficulty the entire membrane could be removed intact.

When samples from the above cultures were examined microscopically, it was observed that the organism had responded to each nutritional deficiency with a distinctly different morphology. As one would expect from the lack of response to any of the vitamins except biotin, omitting biotin alone had the same effect as omitting all the vitamins. The growth on each medium consisted essentially of oval yeast cells with occasional, rather short, thick mycelial strands (pl. 15, fig. 2). The effect of the lack of sugar could not be differentiated from that obtained on a biotin-deficient medium. In this medium the only carbon source was ammonium citrate which, for this organism, is a very poor one.

Material from the phosphate-deficient medium consisted of very long mycelial strands with comparatively few typical yeast cells and blastospores. The most conspicuous characteristics were the numerous chlamydospores which developed in a very short time and the large vacuoles in the hyphae and yeast cells (pl. 15, fig. 3).

[2] Jones and Peck (1940) have reported a green pigment produced by *Candida albicans* and *C. stellatoidea*.

The potassium-deficient medium also yielded a growth form of a rather distinct morphology. Although there were practically no free yeast cells, neither was there ever a true mycelium. The entire growth consisted of clusters or rosettes of pseudohyphae composed of elongated, distinctly separate cells (pl. 15, fig. 4).

The samples from all the rest of the media were found to be just as much alike microscopically as they were macroscopically. All consisted of very dense entanglements of very long, thread-like, apparently non-septate hyphae. The cultures contained very few yeast cells and blastospores when young, but as they grew older these forms began to predominate (pl. 15, fig. 5). The effect of age will be discussed in more detail in a later section.

As indicated above, the basal medium contains several constituents which are not necessary for good growth. To test these effects further, the following medium was prepared in slants and inoculated:

Sucrose	10.0 gm.	Calcium pantothenate	1.0 mg.
$MgSO_4 \cdot 7\ H_2O$	10.0 mg.	Copper sulfate	25.0 microgm.
$MgSO_4 \cdot 7\ H_2O$	10.0 mg.	Agar	20.0 gm.
Amm. citrate (dibasic)	6.0 gm.	Distilled water to	1000.0 ml.
Biotin	2.5 microgm.		

Growth on the above medium after two days was not as heavy as that obtained on the complete basal medium, though the morphology was the same. Since it was desired to obtain the best growth possible, a basal medium was prepared, consisting of all of the heretofore-mentioned substances, except the vitamins, riboflavin, calcium pantothenate, inositol, thiamine, and pyridoxine, and asparagine.

From the data obtained on the containers of the chemicals used, it was calculated that at least the following quantities of inorganic constituents were present per liter of medium under all conditions:

Magnesium, less than .05 mg.	Iron, .065 mg.
Phosphorus as phosphate, less than .02 mg.	Copper, .0075 microgm.
Zinc, .0075 microgm.	Other sources of trace substances are from the distilled water and the agar.

Undoubtedly, most of these elements, especially magnesium, iron, and phosphorus, are required by this organism, but with the exception of phosphate, these requirements are so low that a demonstration of them is rather difficult. For information concerning the purification of media and the effects of various metallic ions on the growth and metabolism of fungi, the reader is referred to Perlman (1949).

Effects of various carbon sources.—The medium used was the basal medium previously described but with the omission of all of the vitamins except biotin, and the substitution of other possible carbon sources for sucrose. Large Durham fermentation tubes (25 ml. of medium) of the media were prepared, using 5 per cent sugars, and inoculated heavily from a culture previously grown on the complete basal medium. Since the only difference in any of the media was the carbohydrate, the complete medium is designated only by the name of the sugar.

The fermentation was at room temperature (25–30° C.). The results are given in Table III.

TABLE III

ACID AND GAS PRODUCTION BY *CANDIDA ALBICANS* A.T.C.C. 2091 IN SYNTHETIC CARBOHYDRATE MEDIA

Carbohydrate	Reaction	Gas
Glucose	Acid	++++*
Fructose	Acid	+++
Mannose	Acid	+++
Galactose	Acid	+
Maltose	Acid	++
Sucrose	Acid	—

*Relative rates of gas production are indicated by the number of plus signs; — indicates no gas production.

In addition to fermentation tests, agar slants of the same basal medium were prepared using the above sugars as well as alcohol, glycerol, starch, and succinic acid as sole sources of carbon and in combination with each other. Various quantities of each compound were used to determine the effect of concentration. These media were inoculated and incubated at 24° C. for 24 hours.

Results.—Fermentation (gas production) did not become apparent in glucose medium until after two days and not in the others until several hours later. By the end of three days, both the glucose and mannose fermentations were quite active with considerable gas production. There was a small amount of gas in the fructose fermentation tube, but in the galactose, maltose, and sucrose tubes, there was still no activity. Eventually, the maltose fermentation was active and still later the galactose, but there was no gas production from sucrose after a month.

On slants containing the above medium, with 2 per cent concentrations of glucose, fructose, and mannose respectively, there was no noticeable macroscopic difference in growth. All cultures grown on these media, after one day, were soft, creamy white, and very easily removed from the slant with a wire loop. Microscopic examinations of all three of these cultures revealed a complex mixture of yeast cells and filaments (pl. 15, fig. 6).

The cultures grown on galactose, maltose, and sucrose media were almost white, rather dry, and so tough that a wire hook was required to tear portions from the slants. The growth on the galactose culture appeared somewhat more luxuriant than that obtained on the other media, and after a few days it became quite pubescent and yellow in color. Microscopically, the growth on galactose medium was the most purely mycelial of any of the cultures obtained on these three media, although all three were composed predominantly of long, thread-like, non-septate filaments (pl. 17, fig. 18).

Lactose and starch were not utilized by this organism, and the resulting growth was like that obtained on medium containing only citrate as a carbon source. The reactions of these media became alkaline.

Alcohol, glycerol, and succinic acid were utilized but the growth was almost entirely yeast-like (pl. 15, fig. 2). Two per cent succinic acid produced the richest growth of the three. These media became alkaline in reaction.

In all cultures in which 1 per cent alcohol was added to the sugar media, the growth became more filamentous than that obtained on sugar media alone. When 3 per cent alcohol was added, however, the resulting growth was characterized by rather large yeast cells in clumps and chains, with very frequent, rather short, pseudohyphae attached, presenting many clavate structures (pl. 16, fig. 7). When 5 per cent sugar concentrations were used, there was greater tendency in all media to the yeast-like phase. Growth resulting on galactose medium was still the most mycelial of the group.

All the above cultures were examined from time to time both macroscopically and microscopically to determine the effect of age. Microscopic examination never revealed a culture which became more filamentous with age. However, to the naked eye some of the cultures, particularly those which on the first day were practically pure mycelial structures, became more hirsute after four or five days longer incubation. Those apparently thread-like strands seen by the unaided eye extending out from the edge of the colony were each composed of a single long filament very thickly covered with dense clusters of blastospores. The central filaments were too small to be seen macroscopically, but there is little doubt that they had been produced long before they could be noticed. Microscopically, any sample taken from a culture, which had been almost entirely mycelial when only one or two days old, after several days revealed a preponderance of yeast-like cells. The mycelium which was still present was apparently devoid of protoplasm since it would not stain except for occasional granular structures. It was observed that the yeast cells developed between the membranous mat produced by the mycelium and the agar, and eventually broke through to the surface as the membrane degenerated. A culture which was yeast-like at the beginning was never observed to become more filamentous with age.

Influence of Temperature

Media.—The medium used in determining the effect of temperature upon filamentation was mostly the original basal medium, but almost every other medium used in this study was tested at various temperatures at one time or another. Agar slants were inoculated in triplicate, and one of each was incubated at 24° C., 37° C., and 40° C., and examined at 24- and 48-hour periods.

Results.—On media which were previously found to produce the yeast form at 25°–30° C., there was little difference in either the macroscopic or the microscopic appearance resulting from the three incubation temperatures. In the media which had been previously found to favor a mycelial form at room temperature

there was considerable difference. Grown at 24° C., these cultures were tough and membranous; at 37° C. and 40° C., they were no longer tough and membranous but soft and creamy. Microscopic examination revealed in those cultures incubated at 24° C. very thin, thread-like filaments with few yeast cells and blastospores (pl. 16, fig. 8), and in those incubated at 37° C. and 40° C., thick, septate pseudomycelium and rather large yeast cells in rosette-like clusters (pl. 16, fig. 9). Their general appearance is much more yeast-like than those incubated at lower temperatures.

Influence of the Consistence of the Medium

Methods.—A series of liquid media was prepared as previously described but with each medium lacking one essential nutrient. Tubes of these media and of the complete medium were inoculated in triplicate, and one of each was incubated at 24° C., 37° C., and 40° C. After two days' growth, the cultures were examined macroscopically, then they were shaken to provide uniform sampling. Samples were taken with a long, dropper-type pipette and mounted on slides for microscopic examination.

Results.—Heaviest growth occurred in the complete basal medium at all temperatures. There was considerable turbidity in the upper part of the medium and a flocculent sediment at the bottom. In the biotin-deficient medium there was a flocculent mass at the bottom of the tube and no turbidity in the upper part. In cultures from which sugar was omitted and also in those from which phosphate was omitted, there was a flocculent mass which settled rapidly when the tubes were shaken. Cultures lacking potassium were very granular and settled rapidly after they were shaken. Temperature had no visible effect upon gross morphology.

Microscopically, the differences which were obtained on different liquid media at different temperatures were not so distinct as they were found to be on solid media. This was especially true of the cultures on biotin- and sugar-deficient media, which were much more mycelial than corresponding agar slant cultures. The potassium- and phosphate-deficient media produced forms like those produced on agar slants of these media. The pseudomycelium consisting of rosettes of yeast-like cells were equally noticeable in the potassium-deficient medium. The other cultures consisted of long, thread-like filaments with numerous blastospores and yeast-cells.

There was little noticeable difference in cultures incubated at 37° and 40° C. Although high temperature inhibited filamentation on the slant cultures, this factor had surprisingly little effect on liquid media. The filaments became, perhaps, a bit thicker with more of a pseudomycelial tendency.

Tubes of the above media were also inoculated and aerated for two days by bubbling air through them. These were little different from those described for the non-aerated cultures above.

In general, most of the differences noted on agar slants were present in liquid cultures but they were less distinct. The pure mycelium and yeast forms obtained on agar slants were not obtained in liquid media.

Effect of anaerobism.—Mycologists generally agree that filaments are produced as a result of reduced oxygen tension, this conclusion having been reached mainly through comparison of growth in liquid and solid media. Since filaments were found more common in liquid media than on solid, they consider the difference to be due to the difference in oxygen available to the organism under the two conditions. Wickerham and Rettger (1939), however, described the growth of *Candida albicans* on corn meal agar under what they considered reduced oxygen tension, being accomplished by placing a cover glass over a developing colony on a petri dish or on a slide covered with a thin layer of agar. Langeron and Talice (1932) found that carbon dioxide had a stimulating effect on mycelium production. In order to test further the effect of anaerobic conditions, the following experiment was performed.

The chemical reservoir of a large desiccator (21-liter capacity) was filled with 10 per cent sodium hydroxide solution. Two petri plates and two agar slants containing sucrose basal medium were inoculated by heavy streaking from a similar culture. Approximately 150 gms. of pyrogallic acid were mixed with the sodium hydroxide solution. The cultures and a lighted candle were then introduced into the desiccator and the lid replaced. To insure sealing, the lid and rim of the desiccator were well greased with stopcock grease. For controls, like cultures were prepared and incubated outside the desiccator. All were incubated at room temperature for two days before examination.

Results.—The rich growth of the cultures incubated outside the desiccator and the almost complete lack of growth of those incubated inside the desiccator indicated that anaerobic conditions had been achieved. When material from all cultures was examined microscopically, there was little detectable difference. In both cases there were long filaments mixed with blastospores and yeast cells. The anaerobic cultures contained rather large vacuoles. When the cultures that had been incubated anaerobically were placed under aerobic conditions, they soon developed abundantly. Although all the plates had been uniformly streaked over a rather large area, most of the growth was at the edges, so that a thick widening ring was formed around the outside (pl. 16, fig. 10). A halo of hyphae surrounded the outer edge of the ring, but there were few within the surrounded area. This phenomenon is undoubtedly the same as that described and photographed by Magni (1948) in his work on reciprocal inhibition of pseudomycelium formation in parallel colonies. He believed that the lack of pseudomycelial development between parallel colonies was due to the lack of nutrients.

Effects of Various Other Substances

Various factors, in addition to those just discussed, have been reported to influence the morphology of this organism. Negroni (1935) reported the influence

of phenol in producing a rough (R) type colony of *Candida albicans* approximating the R type colony of bacteria. Mickle and Jones (1939) studied the effect of lithium chloride and immune serum on dissociation. Nickerson and Jillson (1948) found that the mycelial phase of *Candida albicans* was completely inhibited by culture filtrates of *Trichophyton rubrum*. Varying concentrations of beta indole acetic acid were found by Morquer and Nystérakis (1948) to be very influential in bringing about a filamentous form. Langeron and Guerra (1939) reported the influence of so-called "elongation factors" chief of which are high concentration of carbon dioxide and substances of high molecular weight such as peptone, and nitrates. Nickerson (1950) noted an inhibiting effect of cobaltous nitrate and proflavine on cell division in *C. albicans* with the consequent production of the mycelial form. According to him, .001 M cysteine not only inhibits chlamydospore and mycelium formation (which he considers are brought about by the same factors) in his basal medium, but also counteracts the effect of cobaltous nitrate and proflavine. Most authors believe that a high carbon-low nitrogen ratio is also conducive to mycelium production.

Certain of the experiments were repeated in this study with varying degrees of success as will be indicated.

Methods.—The medium used was usually that described above, but peptone and yeast extract agar were sometimes used. Sucrose, glucose, and galactose were used as carbon sources. All of this particular phase of work was done on agar slants.

Effect of phenol.—Galactose basal media containing approximately .05 per cent and .1 per cent phenol were inoculated with *C. albicans* and incubated at 24° C. for 24 hours. Samples were taken from the slant and prepared as previously described for microscopic examination.

Results.—Macroscopically, both the above cultures were rather rough, somewhat granular, and soon became brown in color. Microscopically, these cultures were observed to consist of very thick, irregular pseudohyphae and large yeast cells. No chlamydospores were observed (pl. 16, fig. 11).

Effects of cobaltous nitrate and cysteine.—The following media were inoculated with *C. albicans* and incubated at 24° C.:

1. Basal medium less all vitamins except biotin; 2 per cent galactose; .05 per cent cobaltous nitrate.
2. Medium as above except $MgSO_4$ was increased five fold.
3. Medium like No. 1; 2 per cent sucrose; .001 M cysteine.
4. Medium as above; 2 per cent sucrose; .001 cysteine; .05 per cent cobaltous nitrate.
5. Medium as above; 2 per cent sucrose; .002 M cysteine.
6. Medium as above; 2 per cent succinic acid; .05 per cent cobaltous nitrate.
7. Yeast extract, 1 per cent; sucrose, 2 per cent; KH_2PO_4, .02 per cent; $Co(NO_3)_2$, .05 per cent.
8. Yeast extract, 1 per cent; sucrose, 2 per cent; KH_2PO_4, .02 per cent; $Co(NO_3)_2$, .1 per cent.
9. Peptone, 3 per cent; galactose, 2 per cent; KH_2PO_4, .02 per cent; $Co(NO_3)_2$, .05 per cent.
10. Peptone, 3 per cent; glucose, 2 per cent; KH_2PO_4, .02 per cent; $Co(NO_3)_2$, .05 per cent.

Results.—The above cultures were examined at the end of twenty-four hours and from time to time thereafter. In the 24-hour cultures, there were little

macroscopic or microscopic differences between Nos. 1, 2, 3, and 5 or the basal media, using the corresponding carbon sources with the cobaltous nitrate and cysteine omitted. The basal media containing sucrose and galactose as carbon sources produced a very mycelial form when cobaltous nitrate was present. Somewhat later those containing cobaltous nitrate became rough, rather granular and dry. Culture No. 2, with a high content of magnesium sulfate, remained more like the cultures previously described on basal medium.[3] Culture No. 6, using succinic acid as a carbon source, was composed almost entirely of yeast cells, and no difference could be detected due to the addition of cobalt. Cultures 7, 8, 9, and 10 were much alike but greatly different from cultures grown on basal medium or on yeast extract or peptone media not containing cobaltous nitrate. In these natural media, cobaltous nitrate showed a definite growth inhibition not noted on the synthetic media. Growth developed very slowly on these media, beginning in small granular, brownish colonies on the thin part of the slant and slowly spreading down until, after several days, the whole slant was covered. Microscopically, these cultures were observed as clumps and chains of yeast-like cells. These last four media where cobalt nitrate was omitted gave complicated mixtures of mycelium and yeast cells.

The addition of cysteine seemed to have a slight toxic effect in the concentrations used, but it did not entirely prevent filaments from forming. When cysteine and cobalt nitrate were used, the organism became more yeast-like than when either was used alone, but this would seem to be due simply to the increased concentration of toxic substances. Chlamydospores were soon observed in these cultures when one or the other or both of these compounds was added to the media. At the concentrations used in these experiments, cysteine and cobalt had little morphological effect except for slight toxicity as indicated by the roughness of the cultures and decreased growth in certain cases.

Effects of other chemical substances.—No detailed studies were made on the other supposedly influential factors previously listed. The increased concentration of peptone to 5 per cent increased mycelial production as reported by Langeron and Guerra. Substances of high molecular weight were not tried as causes for filament production, since they were not employed in the medium which gave an almost pure mycelium. The highest molecular-weight compound used in this medium, other than the sugar, was ammonium citrate, and it was found that ammonium chloride gave an equally good mycelium and approximately the same amount of growth. Since nitrates were not employed at all, it is concluded that a good mycelium can develop in their absence. As for the necessity of a high carbon-low nitrogen ratio for mycelium production, it was found that when the carbon source was raised to a higher concentration than 5 per cent, there was a great tendency toward the yeast form.

[3]This supports the findings of Abelson and Aldous (1950) concerning the antagonism of cobalt and other bivalent ions toward magnesium metabolism. They found that nickel and cobalt were less toxic to a variety of microorganisms when the magnesium content of the medium was increased.

Chlorides.—Although the effect of chlorides was not studied extensively, the observations made in the course of this work seem to be worthy of a brief remark here and of further study in the future.

While studying the effect of potassium on morphology (KCl being the potassium source) it was observed that increased concentrations of this salt up to 10 per cent would produce a purer mycelium when glucose was used as the carbon source than would the medium containing the normal, comparatively low concentration. Since it had been previously observed that potassium was necessary for the formation of a mycelium, higher concentrations of this element were believed to account for the mycelial stimulation. However, when 10 per cent NaCl was employed, using the normal amount of KCl in a glucose basal medium, this mycelium-stimulating (or yeast- and blastospore-retarding) tendency was observed to be as strong as in the 10 per cent KCl medium.

Morphology on Various Natural Media

Skinner (1947) has listed a number of natural media employed by mycologists in their morphological studies, but he preferred Benham's corn-meal agar as prepared by Bernhardt (1946) and Anderson's corn meal infusion for inducing mycelium and chlamydospore production. Wickerham and Rettger (1939) found corn-meal agar very suitable for true mycelium production. Talice (1930) preferred potato infusion or potato agar for inducing filamentation. Sabouraud agar (glucose peptone agar) has found wide use in morphological studies, giving cells almost exclusively of the budding yeast type. Sugar-free beef peptone gelatine stabs have also been reported useful. Diddens and Lodder (1934) employed a number of natural media, among which the most used were malt extract, wort, wort agar, glucose peptone agar, and milk.

Media.—Various natural media, including Bacto malt extract, corn steepwater, yeast extract, Bacto peptone, Bacto beef, Bacto corn-meal agar, and potato and carrot decoctions, were used alone and in combination with the previously used sugars. In addition, some of these natural substances were added to the complete basal medium. These media, prepared as slants, were inoculated with a 24-hour-old culture grown on yeast extract-glucose agar at 24° C.

Results.—The malt-extract culture was the most filamentous of the group, having long, thread-like filaments with numerous blastospores. The growth of this culture was also quite heavy. Corn steepwater and yeast extract cultures were predominantly yeast-like. When yeast extract was added to the complete basal medium, which ordinarily produces the mycelial form, a yeast-like form was produced. Peptone cultures were always complicated mixtures of filaments and yeast cells. All the above media yielded fair growth, but the addition of mineral salts and sugars usually increased the growth. The Bacto beef and corn-meal agar cultures were fairly filamentous. However, most of the filaments were rather short, and in young cultures were swollen at the ends. These cultures showed very poor growth even with sugars and potassium phosphate added. The growth

on potato and carrot agar was quite good, being of the soft, creamy type. Both these cultures contained many pseudohyphae and a preponderance of yeast cells. The addition of sucrose to these media improved the growth, but the morphology was virtually unaffected. There was little better growth, if any, in any of these media than that obtained on the basal medium. In most cases it was inferior.

Morphological Comparisons of the A.T.C.C. Strain 2091 With Other Cultures of *C. albicans*

Five cultures of *Candida albicans* were obtained from Dr. Mackinnon which were without data except for the initials and numbers used to designate the individual strains. These cultures were designated as 1. H.M. 493, 1. H.M. 805, 1. H.M. 806, 1. H.M. 679, and 1. H.M. 582. Agar slants of galactose basal medium from which all vitamins except biotin were omitted were inoculated with these strains and were incubated at 24° C. for 24–48 hours. The cultures were then examined macroscopically and microscopically.

The slant culture 1. H.M. 493 was almost pure white, rather soft, and wrinkled. Microscopically, it was quite mycelial, but the hyphae were rather thick and twisted, indicating that, although the growth was quite heavy, the medium was not altogether suitable for the best growth of this organism (pl. 17, fig. 13). Culture 679 was rough, cream-colored, and quite soft. Microscopic examination revealed a fairly good mycelial growth and many somewhat lance-shaped yeast cells. Growth was good (pl. 17, fig. 14). Culture 805 did not grow very well on this medium. The growth appeared rather dry, almost white, and was easily removed from the slant with a wire loop. Microscopically, it was observed to be a mixture of yeast cells and pseudohyphae (pl. 17, fig. 15). Slant culture 806 was a very heavy, almost white, velvety growth and so tough and membranous that a wire hook had to be used to remove material from the slant. As one would expect from such a membranous material, this culture was observed microscopically to be very mycelial. The individual cells present were very narrow and rather long (pl. 17, fig. 16). Culture 582 was of a very soft, creamy, glistening white material. Growth was very rich. Microscopically, this culture was seen to consist preponderantly of small yeast cells, but there were occasional long, thread-like hyphae (pl. 17, fig. 17). Although chlamydospores are not shown in the photograph, they were later observed to occur frequently in chains of six or seven as well as individually at the tips of filaments.

The six strains of *C. albicans*, including the five Mackinnon strains and the A.T.C.C. strain 2091, differ quite distinctly in their morphologies when grown on the same medium at the same time under identical conditions. Not only are the tendencies to become yeast-like or mycelial different in degree, but the individual yeast cells and blastospores are different in shape and size. The yeast cells of cultures 582 and 805 resemble most closely those of the A.T.C.C. culture, but their mycelial tendency on galactose basal medium is less pronounced. The mycelial growth of

culture 806 is greater than is ordinarily obtained with the A.T.C.C. culture, and the blastospores and individual cells are more slender and much longer. Cultures 493 and 679 resemble the A.T.C.C. culture grown under adverse conditions. Previous morphological and physiological relationships observed on the latter strain would indicate that these organisms also have different physiological requirements.

Discussion

It is evident from the results obtained in this study, at least so far as this particular organism is concerned, that some of the factors affecting morphology given by previous authors must be somewhat modified. For the sake of clarity and convenience, these factors will be considered individually.

Influence of pH.—From the review of the literature there seems to be little agreement among the various workers concerning this factor. To the extent that extreme pH ranges exert a toxic effect which has a morphological influence on the organism, the results of this study are in agreement with those of Roux and Linossier (1890). These workers found that the toxic effect is manifested by an individualization of filaments. In the present study, however, the toxic effect of extreme pH ranges, as well as other types of toxicity, almost invariably produced yeast-like cells. As previously discussed, Talice (1930) considered this factor rather important, but that the most filamentous morphology is obtained at pH 8. According to Langeron and Guerra (1939), pH is one of the most important factors, filaments being produced in an alkaline medium, yeast cells in acid.

Since there were no precise methods employed in this study for determining relative rates or quantities of growth, the exact pH optimum is not certain. The most regular, thread-like filaments and uniformly oval yeast cells and blastospores were produced at pH 5. Increasing or decreasing the pH resulted in swollen, irregular filaments, a preponderance of yeast-like cells, and an early (2 days) appearance of the thick-walled chlamydospores. This irregular morphology having been observed constantly in media known to be unsuitable for optimum growth, it is concluded that a slightly acid range (pH 5–6) is optimum for this organism. It is thus evident that pH is a very important factor, though the range must be varied considerably to exert a very noticeable influence. This influence is probably due to the toxicity exerted upon the organism. It is, perhaps, noteworthy also that the medium soon becomes acid when a readily assimilable carbohydrate is employed. When a carbon source not so readily assimilable is used or the source is too dilute, the medium becomes alkaline. It is considered that the real, morphology-determining factor in this case is one of nutrition, but the pH changes probably have some influence also.

Influence of Nutrients.—It is generally agreed among mycologists that this particular factor is of prime importance and that filamentation occurs as a result of starvation. The idea that "impoverished" media is necessary for the production of filaments developed as a result of growing the organism on various natural

substances of unknown chemical composition. It is apparently true that most natural media which produce the filamentous form yield a rather poor growth, whereas those which produce a yeast form usually yield a heavier growth. The results obtained on natural media in this study agree with those of previous authors. The results obtained on chemically identified media, however, do not support the general statement concerning "impoverished" media and are in direct opposition to that concerning the morphological influence of readily assimilable carbohydrates.

Of the sugars used in this study, galactose gave the heaviest mycelial growth, and maltose and sucrose were better than glucose, fructose, and mannose. With the exception of sucrose, which was never fermented (gas), there seems to be a relationship between the rate of fermentation and the amount of filamentation. Those which were most readily fermented (glucose, fructose, and mannose), though producing abundant filamentation, also produced more blastospores and yeast cells than the less readily fermented sugars. The reducing sugar content within the range of 1 to 3 per cent does not appear to have the importance in cell division that Nickerson attributed to it. Galactose, also a reducing sugar, not only produced the most abundant growth, but also the most abundant mycelium.

It has been shown that good filamentation not only can occur on fairly large concentrations of readily assimilable carbohydrates, but that they are necessary for good filamentation. In addition to the necessity of carbohydrates, potassium and biotin are also essential. An absence or deficiency of any one or all of these three substances results not only in a very poor growth, but the growth which does occur is of the soft, creamy type of yeast-like morphology. Phosphorus, though essential to the growth of the organism, does not seem to affect its filamentation to a very great extent. With more highly purified chemicals than ordinary C. P. chemicals such as those used in this work, the effect of phosphorus, as well as some of the other minor elements, would undoubtedly have been more evident. The very noticeable effect of the phosphate deficiency was the very early (24 hours) appearance of numerous chlamydospores. The fact that no other deficiency produced this effect in such a short period of time indicates that the production of chlamydospores is stimulated by the exhaustion of the available phosphorus in the medium. Another obvious feature of the organism grown on phosphate-deficient medium are the numerous, large vacuoles both in the filaments and the yeast cells.

Many mycologists have observed that natural media can be divided into two groups depending upon whether they produce a yeast-like or a filamentous growth of *Candida albicans*. It has been shown in this study that those substances which produce a filamentous, though a poor growth, can be fortified with carbohydrates and inorganic salts to produce good growth without affecting the morphology of the organism—that is, a heavy filamentous growth. On the other hand, no amount of fortification has been found suitable for inducing a yeast-producing natural medium to produce filaments. When a yeast-producing substance such as yeast

extract is added to a complete synthetic medium which produces abundant filamentous growth, the resulting growth is soft, creamy, and yeast-like, but little heavier than that obtained on synthetic medium alone. The results of these experiments indicate that most natural media contain various unknown substances which induce a yeast-like morphology in *Candida albicans*. That there is ample available carbon in these substances is shown by the rich growth which occurs upon them without additional carbon sources. It is doubtful that these substances are sugars since the metabolism of the organism brings about an alkaline reaction instead of the characteristic acid of carbohydrate metabolism. It is perhaps true that these natural media may contain so much nitrogenous material that the ammoniacal products of metabolism may mask the acidity given off by the carbohydrate metabolism. However, one pure natural substance, succinic acid, was readily utilized as a carbon source, and the medium became alkaline. The resulting morphology on this medium was yeast-like. There are doubtless other substances in natural material which serve as carbon sources for this organism and produce the yeast-like form.

It is then necessary to modify or perhaps do away with the term "impoverished" media when referring to media necessary for producing mycelium in *Candida albicans*, since a filamentous growth can also be a very rich growth.

Influence of temperature.—Except in liquid media where there was little detectable difference, a high temperature (37–40° C.) produced a very strong tendency toward the yeast phase. The only explanation for the discrepancy between this finding and that of other authors is that we are evidently using different organisms. If this be true, then a better description of the organism is needed, since the characteristics of this one have fulfilled all the morphological and biochemical requirements listed by the taxonomists.

Effect of the consistence of the media.—It has been observed, almost from the first study made on this organism, that the mycelial tendency is stronger in liquid than on solid media. We found this especially true in a medium which usually produced a yeast-like morphology in the solid state. The other factors, such as temperature and even nutrition, were not so obvious in their effects, though they were usually noticeable. This effect is generally attributed to the reduced oxygen tension in liquid media, but it is not so easily proven. In this study it was found that the organism could not grow anaerobically on agar. In liquid media the growth seems to occur mainly at the top and then precipitates to the bottom in a cottony mass. Indeed, if one is careful not to shake the culture tube, the mass of the organism is seen to be located in two separate places—one very fine mass at the top and the characteristic cottony mass at the bottom. The liquid between these two masses is often practically clear. By means of a dropper-type pipette, samples of each were obtained separately for microscopic examination. The examination of young cultures revealed short, highly branched chains of yeast cells at the top and long thread-like filaments at the bottom. From these experi-

ments, it seems that filaments produce clumps at the top of the medium which settle to the bottom, leaving space for individual yeast cells or blastospores to begin the process over again. The little clumps of pseudomycelium seem able to grow for a short time after sinking further into the medium, producing the typical filaments. Regardless of what the true process is, the growth cycle is not essentially different from that obtained on solid media. The filaments are produced in abundance only in young culture, and as the culture ages, the filaments degenerate until the culture becomes a granular mass composed almost entirely of yeast cells.

The effect of solid media is just as difficult to interpret as that of liquid media. Wickerham and Rettger (1939) believed that placing a cover-slip over a developing colony created the reduced oxygen tension necessary for filament formation. However, we observed the zone of filamentation consistently on the outer edge of a developing colony on petri-dish cultures which were not covered with cover-slips. Observations on a giant colony reveal that the spread is accomplished by this ever-widening zone of naked filaments which soon become covered with blastospores but never covered all the way to the tips (pl. 16, fig. 12). If reduced oxygen tension favors the production of filaments and retards the production of blastospores, it is rather strange that practically all the filamentous growth is toward the outside of a colony while blastospores are produced nearer the center where competition for oxygen would be much greater. This may be observed in the samples taken at various distances from the center of a giant colony, and the effect is even more striking when a two-inch square of an agar plate is evenly streaked with a culture of *Candida albicans*. The inner zone contains practically nothing except yeast cells, while the outer zone grows like a giant colony producing a luxurious, filamentous growth upon which blastospores develop (pl. 16, fig. 10).

The above descriptions are typical of growth obtained on good filament-producing media. When a poor mycelium-producing medium is used such as succinate basal medium the results become confusing. The growth on slants, as previously observed, is almost entirely yeast-like with only occasional filaments. In a giant colony, though the center is yeast-like as expected, there is also an outer zone of filaments. However, instead of being on the surface as they are in the carbohydrate basal medium, all seem to be growing down into the agar. They become covered with a sleeve of blastospores which makes them visible macroscopically. It is believed that this phenomenon and those previously described in liquid media have led to the conclusions in regard to anaerobism.

The relationship between the ability of the organism to produce filaments and its ability to produce gas (anaerobic fermentation) on a particular substrate should also be considered. In every case there was better mycelium production on those sugars (galactose, maltose, and sucrose) which were fermented very slowly than on those (glucose, fructose, and mannose) which were rapidly fermented. Also, in five tubes each of glucose and sucrose broth inoculated with one loop of suspension from the same inoculum and incubated in the same rack, the sucrose cul-

tures could quite easily be distinguished from the glucose because of their more abundant growth. This indicates not only that anaerobic fermentation fails to help in the production of a mycelium, but it also lowers the efficiency of the sugar utilization. From my observations it is therefore concluded that, with the proper medium and incubation at the proper temperature, comparable results are obtained on liquid and solid media.

Effects of adding various substances to the basal medium.—In general, substances not required by the yeast but which influence its morphology are of two types: (1) those which show their toxicity by retarding growth; and (2) those which do not appreciably influence the quantity of growth but influence the morphology of the organism.

There are, of course, numerous known chemicals of the first type—phenol, various metallic ions such as cobalt, etc., if used in too high concentrations, and anions such as iodide and chloride. Their toxic effect on morphology is nearly always toward the yeast form but there are evidently exceptions. High concentrations of chlorides were found, in this study, to inhibit growth somewhat and also seemed to inhibit the development of blastospores so that a purer mycelium was obtained. This may have been the result of the high osmotic pressure exerted by these salts. High concentrations of sugar, however, have the opposite effect on morphology. Nickerson (1950) found that he could suppress the yeast cells and obtain cultures of almost pure mycelium with dilute concentrations of cobaltous nitrate. The second type of substances are chemically unidentified compounds contained in varying amounts in most natural media. The chemical separation and identification of these substances are not within the scope of this study, but their presence is easily demonstrated by adding a bit of natural material such as yeast extract to a complete basal medium and observing the change in morphology exhibited by the organism.

Morphological comparisons of various strains.—If one is to accept all of the strains of yeast-like fungi that various taxonomists have placed in the species *Candida albicans*, he must accept also a great variety of morphologically and probably biochemically different characteristics of the organism. Considering that there are only three criteria upon which one can base his classification—namely, production of terminal chlamydospores; fermentation of glucose, fructose, mannose, and maltose, but not sucrose; and production of filaments—there is little wonder that he is unable to choose any typical organism for his study and have the results agree with those of another mycologist supposedly working with the same organism. Mackinnon (1940) would explain most of these differences as being due to spontaneous variation or dissociation, so that if pure yeast cells are chosen as one extreme and pure filaments as the other, a given strain may have undergone any amount of dissociation which would determine its yeast to filament ratio. If one accepts this as the cause for the differences in all the so-called "strains" of *Candida albicans*, he must also accept the fact that the shapes of the yeast cells and blastospores change considerably. Of the Mackinnon strains, there were at least three

different cell shapes. That these strains were not all satisfied nutritionally is indicated by the swollen, knobby appearance of the filaments. Perhaps, if all of these strains were derived in his laboratory from the same culture, the organism would be so protean that it is impossible to attribute to it any more than the three characteristics given.

With the organism employed for this particular study, the results do not indicate that it is as variable as indicated by Mackinnon. It is true that when this organism was streaked on plates, there were often the two types of colonies described by Mackinnon—the prickly, firm colony that could only be removed intact and the soft, creamy colony. When these aged, however, or were broken up and transferred to slants, there was little difference in their macroscopic or microscopic appearance. Either the filamentous form or the yeast-like form of each was obtained, depending upon the medium upon which they were cultured. The requirements for filamentation were the same for each and the blastospore shape never varied.

Finally, it is observed that an organism is better characterized after the second or third transfer on a given medium in 24- to 48-hour periods. The first transfer, in many cases, does not usually produce an organism greatly different from that upon which it was previously growing, particularly if one does not wash the inoculum thoroughly before using it. It is well known that microorganisms store up some critical materials, especially certain vitamins or growth factors, in sufficient quantity to suffice them for one or two generations on media lacking these elements. The first generation, therefore, may indicate not only the effect of that particular medium, but also that of the stock medium. There is another good reason for two or three successive transfers, if one wishes to study the organism under maximum conditions. The lag phase is virtually eliminated by such frequent transfers, and the organism is maintained at its maximum growth rate.

Summary

In order to determine what factors were influential in determining the morphology of the highly variable *Candida albicans*, a chemically defined medium was utilized. Since this medium was readily modified in various specific ways, it was possible to attribute any morphological change to a definite change in the culture conditions. By varying not only the constituents of the medium, but also the physical factors such as temperature and consistency, quite definite conclusions could be reached. In general, it was found that *Candida albicans* A.T.C.C. 2091 requires for filament production a readily assimilable, but not so readily fermented carbohydrate. It also requires phosphorus, potassium, and biotin. The optimum temperature for filamentation is 25–30° C. The optimum pH is near 5. Filaments are produced most abundantly during the maximum growth phase.

The yeast-like phase results from lack or deficiency in any of the above nutrients, a high temperature (37–40° C.), especially on solid media, unfavorable pH range, and toxic substances. Many natural substances contain unidentified

products which, though not growth-inhibiting, produce the soft, creamy, yeast-like form. Yeast-like forms predominate in the lag and decline phases of a culture as the filaments undergo degeneration.

Chlamydospores are produced as a result of unfavorable conditions such as too high or too low pH, deficiency of phosphorus, and to a less extent other deficiencies which are necessary for maintenance of normal growth.

The effects of liquid media on growth, especially as it pertains to reduced oxygen tension, were indefinite. The organism grew poorly, or not at all, in an anaerobic jar on solid media. On liquid media, the growth was observed on top of the medium from whence it precipitated, leaving room for more such growth. Growth on sucrose medium which, if fermented at all, is admitted to be very slow, was considerably better than that obtained on the readily fermented glucose. The sucrose medium in every case produced the greater proportion of filaments.

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Explanation of Plate 15

Candida albicans

Fig. 1. Effect of a high pH (9). Note the numerous yeast-like cells, the chlamydospores, and the few scattered filaments, × 213. Incubated at 24° C.

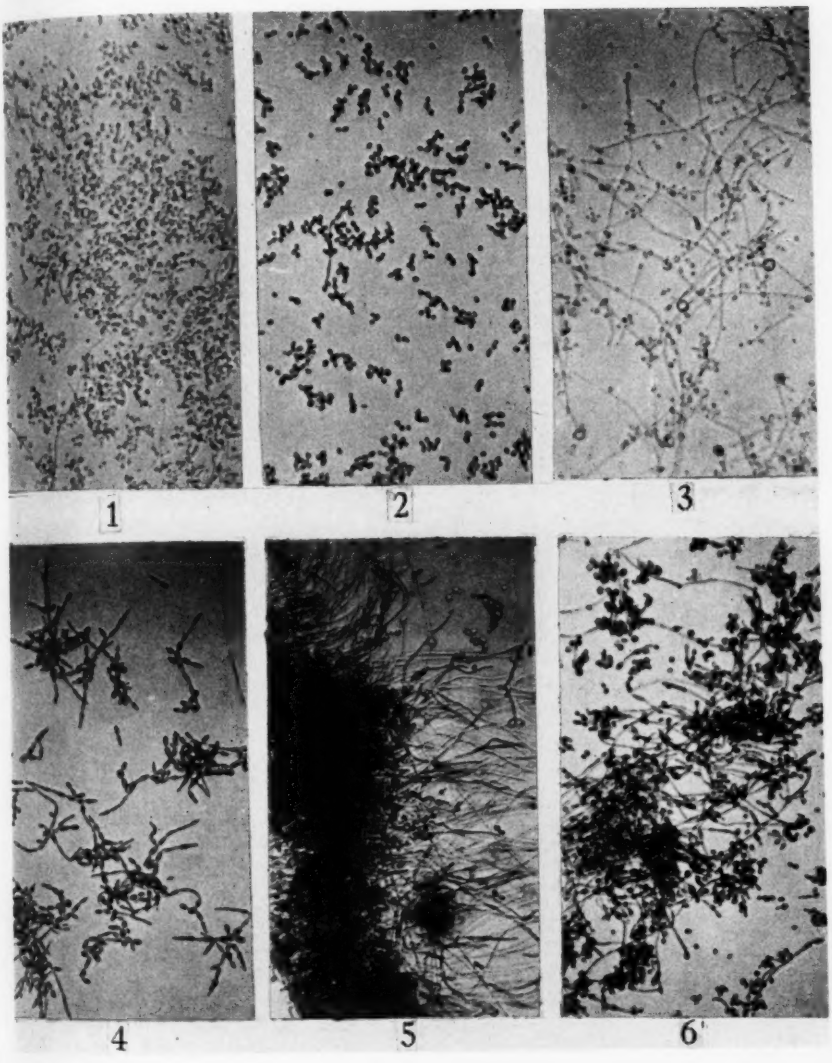
Fig. 2. Growth on approximately 2 per cent succinate basal medium for 24 hours at 24° C., × 213. The same morphology is obtained on histin and carbohydrate-deficient media.

Fig. 3. Growth on a phosphate deficient basal medium 24 hours at 24° C., × 213. Note the fairly numerous chlamydospores and yeast-like cells.

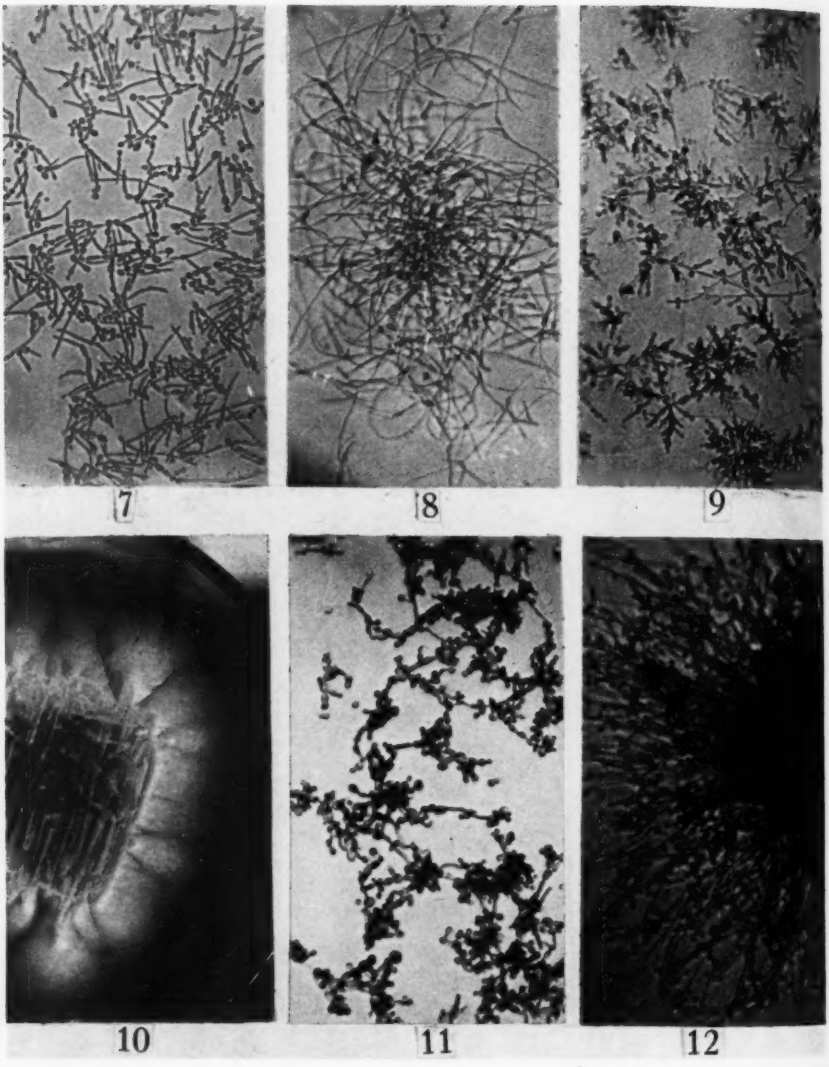
Fig. 4. Rosette-like clusters of short pseudohyphae resulting from a potassium deficiency, × 213. Grown on 2 per cent sucrose basal medium at 24° C. for 48 hours.

Fig. 5. Heavy mycelial growth resulting from growth for 24 hours incubation at 24° C., × 213. Maltose medium produces the same morphology.

Fig. 6. Growth on 2 per cent glucose basal medium for 24 hours at 24° C., × 213. Mannose and fructose media produce the same morphology.



McCLARY—*CANDIDA ALBICANS*



McCLARY—*CANDIDA ALBICANS*

Explanation of Plate 16

Candida albicans

Fig. 7. Culture 24 hours old grown at 24° C. on galactose basal medium containing 3 per cent alcohol, × 213. Much the same morphology was obtained on sucrose and glucose medium containing the same quantity of alcohol, but the filaments were not so long, and typical yeast cells were more numerous.

Fig. 8. Showing effect of a high chloride content in the medium—24 hour growth on glucose basal medium containing 5 per cent potassium chloride, incubated at 24° C., × 213. Compare with fig. 6. Sodium chloride produces the same effect.

Fig. 9. Showing effect of temperature, incubated at 40° C., × 213. Medium and incubation time were the same as for fig. 8.

Fig. 10. A 20-day-old culture on an evenly streaked petri dish, × 820—grown on 2 per cent sucrose basal medium. Note the very scanty, yeast-like growth in the center and the heavy ring at the edge with filaments radiating toward the outside.

Fig. 11. Showing effect of toxic substance, × 213. Growth on basal medium containing .05 per cent phenol.

Fig. 12. Cover-slip culture of a colony several days old which developed from a single yeast cell, × 213. Grown on 1 per cent glucose basal medium at room temperature.

Explanation of Plate 17

Candida albicans

Fig. 13. Mackinnon culture 1. H. M. 493 grown on salts of basal medium, biotin, and 2 per cent galactose, × 213. Incubated for 24 hours at 24° C. Note the twisted, irregular filaments which may indicate that this medium is not entirely satisfactory for this organism.

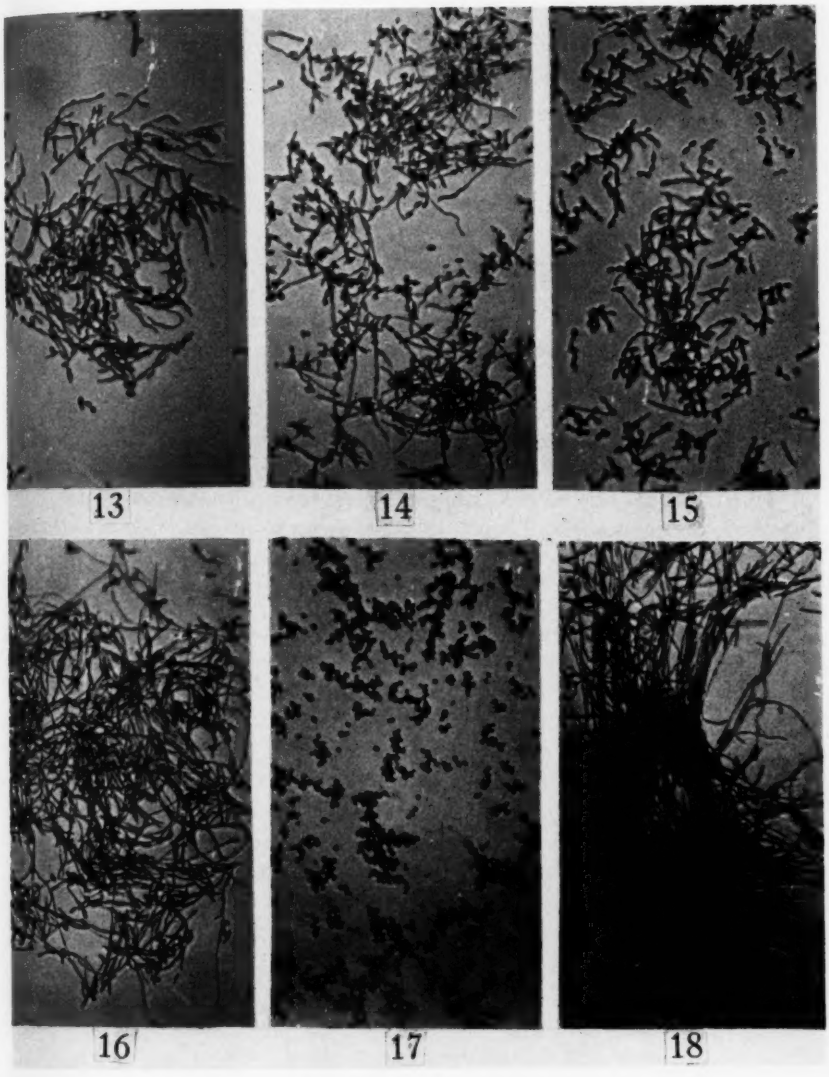
Fig. 14. Mackinnon culture 1. H. M. 679, × 213. Note the rather twisted filaments and the pointed yeast-like cells. Culture conditions identical to the above.

Fig. 15. Mackinnon culture 1. H. M. 805 grown as above, × 213. Note irregular filaments and the long, almost cylindrical individual cells.

Fig. 16. Mackinnon culture 1. H. M. 806, × 213. Note the long, regular filaments and the very long individual cells.

Fig. 17. Mackinnon culture 1. H. M. 582, × 213—almost entirely yeast-like under all conditions tried. Culture conditions here the same as above.

Fig. 18. American Type Culture Collection strain 2091 grown under the above conditions, × 213.



McCLARY—*CANDIDA ALBICANS*

FOREST QUADRAT STUDIES AT THE ARBORETUM AND OBSERVATIONS ON FOREST SUCCESSION

LOUIS G. BRENNER, JR.*

Recently some of the general changes, based on time-lapse studies, occurring in the Forest Preserve of the Missouri Botanical Garden Arboretum, at Gray Summit, were reported on.[1] However, this report covered the Forest Preserve as a whole, and the complex structure of the forest growth was not expressed. Quadrat studies of critical tree associations were begun concurrently with the more general mapping of forest growth, with a view toward acquiring data on the specific changes taking place on smaller, accurately plotted sites which might be expected to lead to an understanding of the problems of forest tree associations in that area. In this paper the changes which have occurred in a lapse of twelve years are reported for three quadrats, and a fifteen-year record is available for one quadrat.

Quadrats, 15 × 15 meters, were selected in areas typical of the several recognized forest-tree associations. All corners of the quadrats were marked with painted iron stakes to insure their accurate location. A grid of stout twine was established at three-meter intervals in order to plot the trees. Approximate trunk diameters (DBH) were measured in inches so that relative dominance of forest species and their growth rate might be recorded.[2]

Quadrat in the Oak (Quercus sp.*) Coppice.*—This quadrat (figs. 1 and 2), representing a 15-year sequence, was established in an oak coppice where stump sprouts indicated that White Oak *(Quercus alba)* was the dominant tree. Soil of this area is of the Union Silt Loam and lies upon the "cotton rock" phase of the Cotter Formation of dolomitic limestones. Exposure is to the east, and the quadrat is near the summit of the ridge. The early map of the quadrat shows a more "open" aspect. At that time abundance of light encouraged the White Oaks to develop a low and spreading crown. The Red Cedar *(Juniperus virginiana)*, Redbud *(Cercis canadensis)*, Walnut *(Juglans nigra)*, Shingle Oak *(Quercus imbricaria)*, Mocker-nut Hickory *(Carya tomentosa)*, and Persimmon *(Diospyros virginiana)* assumed similar growth habits. There were a number of Slippery Elms *(Ulmus fulva)* seedlings, and a small Sycamore *(Platanus occidentalis)* in rather poor condition.

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[1] Beilmann, A. P., and Brenner, L. G. The changing forest flora of the Ozarks. Ann. Mo. Bot. Gard. 38:283–291. 1951.

[2] Species names mentioned in this report are according to Alfred Rehder's, Manual of Cultivated Trees and Shrubs, 2nd ed. 1940.

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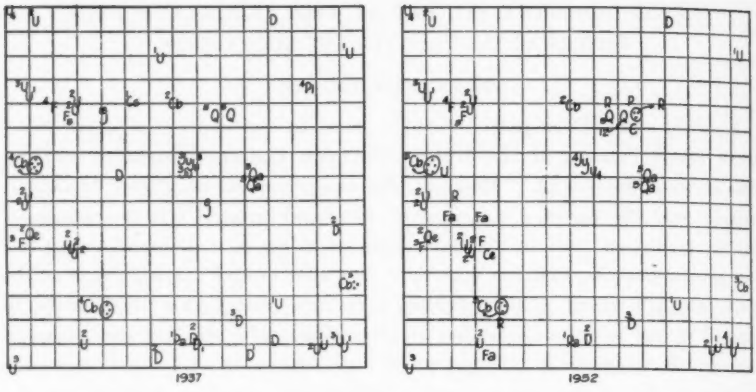

Fig. 1. Plots of a quadrat in the Oak Coppice Association for 1937 and 1952: Q = *Quercus alba*, C = *Carya ovata*, Cb = *Carya tomentosa*, Ca = *Cercis canadensis*, Co = *Cornus asperifolia*, D = *Diospyros virginiana*, F = *Fraxinus americana*, Fa = *Fraxinus quadrangulata*, J = *Juniperus virginiana*, Ju = *Juglans nigra*, P = *Prunus serotina*, Pa = *Prunus* sp., Pl = *Platanus occidentalis*, Qa = *Quercus imbricaria*, Qe = *Quercus velutina*, R = *Rhamnus caroliniana*, U = *Ulmus fulva*. Numerals indicate approximate diameter (DBH) to nearest inch.

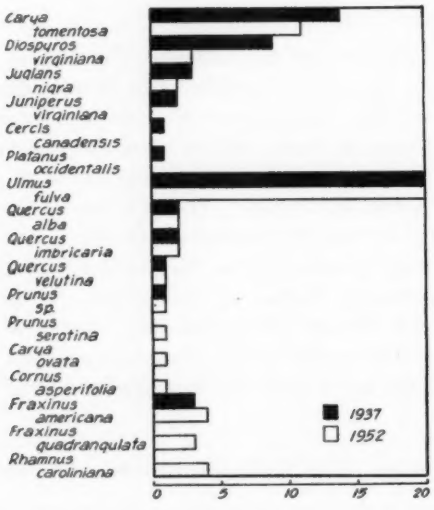

Fig. 2. Diagram representing relative numbers of plants of the species on a quadrat in the Oak Coppice Association in 1937 and 1952.

Recent inspection of the quadrat has revealed a great change in the growth habit of the forest trees. Now the White and Shingle Oaks, Mocker-nut Hickory, and Black Walnut have lost their lower limbs, their trunks are clean twelve to fourteen feet above the ground, and their crowns have developed more spread. The White Oak is still the dominant tree and has made considerable growth. Plants demanding large amounts of light, such as Redbud, Red Cedar, and Persimmon, have mostly been "shaded" out. At least one-fourth of the Persimmons have died and those remaining are in poor condition. The Slippery Elms are no less numerous, but the trees have grown very little. Some seedlings of Shagbark Hickory *(Carya ovata)*, White Ash *(Fraxinus americana)*, Blue Ash *(Fraxinus quadrangulata)*, Black Cherry *(Prunus serotina)*, and Rough-leaved Dogwood *(Cornus asperifolia)* have recently become established in the quadrat.

The record of this quadrat shows how quickly the forest species may become dominant and destroy an "open" aspect. The early land-use history of this area is not clear. It is believed that it had been pastured, and the numerous stump sprouts indicate that some pole-wood had been cut. Pasturing and the cutting of pole-wood promoted the rapid growth of light-loving plants such as Red Cedar, Redbud, and Persimmon, which formed a conspicuous part of the woody growth at the time of the first mapping of the quadrat. Since then and following a more conservative land-use program in which the area has not been pastured or burned, the forest trees have grown so vigorously as to dominate the quadrat area and "shade out" the light-loving plants. The many Slippery Elms, Persimmons, and

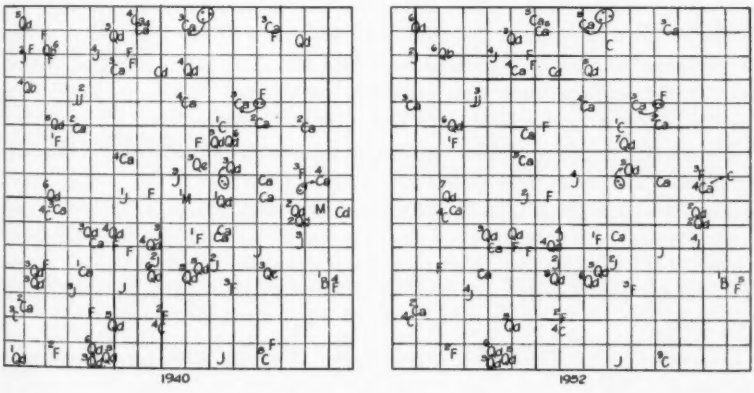

Fig. 3. Plots of a quadrat in the Oak-Hickory Association for 1940 and 1952: Am = *Amelanchier canadensis*, C = *Carya ovata*, Ca = *Carya Buckleyi*, Ce = *Celtis pumila*, F = *Fraxinus americana*, J = *Juniperus virginiana*, M = *Morus rubra*, Qb = *Quercus marilandica*, Qd = *Quercus stellata*, Qe = *Quercus velutina*.
Numerals indicate approximate diameter (DBH) to nearest inch.

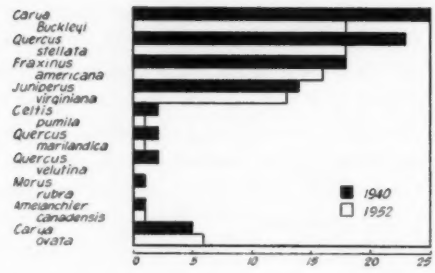

Fig. 4. Diagram representing relative number of plants of the species present on a quadrat in the Oak-Hickory Association in 1940 and 1952.

the Sycamore apparently germinated in the quadrat area about 1924 when it was set aside as a forest preserve. The grassy and otherwise herbaceous ground cover, so conspicuous at the time of the first mapping, has been replaced with duff of forest litter in which seedlings of Shagbark Hickory, White and Blue Ash, Indian Cherry, and Rough-leaved Dogwood have become established.

Quadrat in the Oak-Hickory (Quercus stellata-Carya Buckleyi) Association.—This quadrat (figs. 3 and 4) had been established in an oak-hickory forest just above a glade area. Here the Union Silt Loam overlays a somewhat massive phase of the Cotter Formation of dolomitic limestone. The early map shows small Post Oaks *(Quercus stellata)* and Pignut Hickory *(Carya Buckleyi)* as the dominant trees, and the Red Cedar *(Juniperus virginiana)* and White Ash *(Fraxinus americana)* were also numerous. Other species are mostly represented by seedlings.

The recent map indicates the continued dominance of the Post Oak and Pignut Hickory, but some of these trees have been lost in a natural thinning process. Many of the seedling trees have been lost, along with two large Black Oaks *(Quercus velutina)* and a Black Jack Oak *(Quercus marilandica)*.

The greater numbers of Red Cedar and the numerous seedlings on the early map indicate that more light entered the quadrat twelve years ago. This "open" aspect favored a lower branching habit of all the trees. Now the Post Oak and Pignut Hickory have made considerable growth and support well-developed crowns. They have lost many of their lower branches. Such a closing of the crown canopy has "shaded out" some of the Red Cedars and many seedlings of other trees.

Quadrat in the White Oak-Sugar Maple (Quercus alba-Acer saccharum) Association.—This quadrat (figs. 5 and 6) is located on a lower slope with a western exposure. The soil is the Union Silt Loam overlaying the basal sandstone phase of the Cotter Formation of rocks. The early map shows the White Oak as the dominant tree. The Sugar Maples, though not as large, were then of sufficient

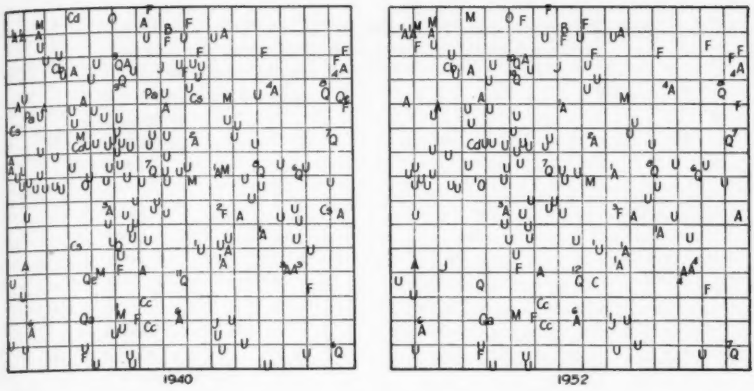

Fig. 5. Plots of a quadrat in the White Oak-Sugar Maple Association for 1940 and 1952: A = *Acer saccharum*, B = *Bumelia lanuginosa*, Cb = *Carya tomentosa*, Cd = *Celtis pumila*, Cs = *Cercis canadensis*, F = *Fraxinus americana*, J = *Juniperus virginiana*, M = *Morus rubra*, O = *Ostrya virginiana*, Pa = *Prunus* sp., Q = *Quercus alba*, Qu = *Quercus velutina*, U = *Ulmus fulva*.

Numerals indicate approximate trunk diameters (DBH) to the nearest inch.

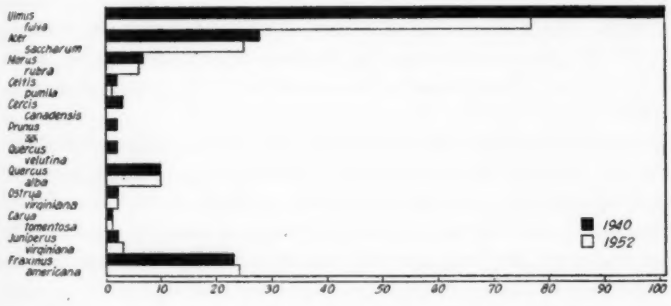

Fig. 6. Diagram representing relative numbers of plants of the species present on a quadrat in the White Oak-Sugar Maple Association.

size and vigor to suggest their co-dominance with the White Oaks. As shown in figs. 5 and 6, the seedlings of Slippery Elm *(Ulmus fulva)* were conspicuous at that time. It is also apparent that there was enough light entering the area to support several Redbuds *(Cercis canadensis)*, as well as Red Cedar *(Juniperus virginiana)*, Dwarf Hackberry *(Celtis pumila)*, and Red Mulberry *(Morus rubra)*. A single Hop-Hornbeam *(Ostrya virginiana)* was thriving.

Recent inspection of the quadrat shows that the White Oak continues to be dominant and that the trees have made appreciable growth. The Sugar Maple is growing slowly and is being suppressed by the White Oak. At least 20 per cent of the Slippery Elm seedlings have been lost and those remaining have made no

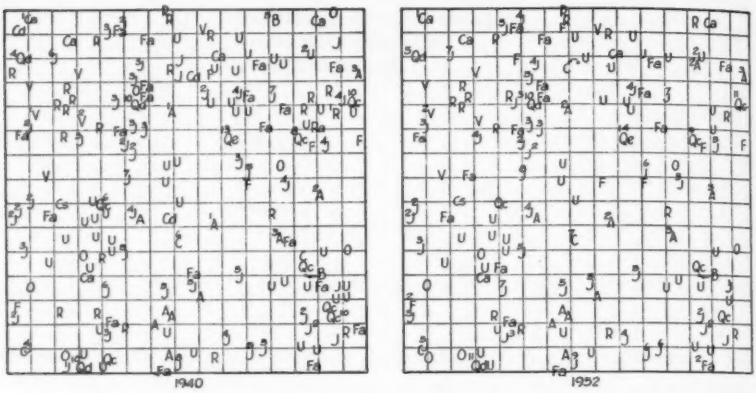

Fig. 7. Plots of a quadrat in the Red Cedar-Chinquapin Association for 1940 and 1952: A = *Acer saccharum*, Am = *Amelanchier canadensis*, B = *Bumelia lanuginosa*, C = *Carya ovata*, Ca = *Carya Buckleyi*, Cd = *Celtis pumila*, F = *Fraxinus americana*, Fa = *Fraxinus quadrangulata*, J = *Juniperus virginiana*, O = *Ostrya virginiana*, Qc = *Quercus Muhlenbergi*, Qd = *Quercus stellata*, Qe = *Quercus velutina*, R = *Rhamnus caroliniana*, Ra = *Rhamnus lanceolata*, U = *Ulmus fulva*, V = *Viburnum rufidulum*.

Numerals indicate approximate trunk diameters (DBH) to the nearest inch.

noticeable growth. The Redbud, Red Mulberry, and Dwarf Hackberry have suffered from reduced light brought about by the expanding crowns of the White Oaks. Red Cedars, though as frequent, have made but little growth and are in poor condition.

The occurrence of old stumps in the area about the quadrat indicates that some trees had been cut prior to the first mapping. Such cutting probably permitted the entrance of enough light to encourage growth of Redbud, Mulberry, Dwarf Hackberry, Red Cedar, and the many seedlings of Slippery Elm. It also may have brought about increased growth of the remaining White Oaks which have become entirely dominant at the expense of the Sugar Maples and seedling trees.

Quadrat in the Red Cedar-Chinquapin Oak (Juniperus virginiana-Quercus Muhlenbergi) Association.—This quadrat (figs. 7 and 8) is located on a lower slope with a western exposure. The soil is very shallow and lies immediately upon the somewhat massive phase of the Cotter Formation of rock. The early map shows a considerable number of Red Cedars 4–7 inches in diameter and a number of Chinquapin Oaks of comparable size. These two species were the dominant trees of the quadrat. Also present were two large Post Oaks *(Quercus stellata)*, a Black Oak *(Quercus velutina)*, and a single large Chittimwood *(Bumelia lanuginosa)*. At that time the quadrat had a "brushy aspect", with Slippery Elm *(Ulmus fulva)* making the greater part of the undergrowth, and in less abundance Redbud *(Cercis canadensis)*, Indian Cherry *(Rhamnus caroliniana)*, Hop-Hornbeam *(Ostrya virginiana)*, Dwarf Hackberry *(Celtis pumila)*, Lance-leaved Buckthorn *(Rhamnus lanceolata)*, Shadbush *(Amelanchier canadensis)*, and Black Haw

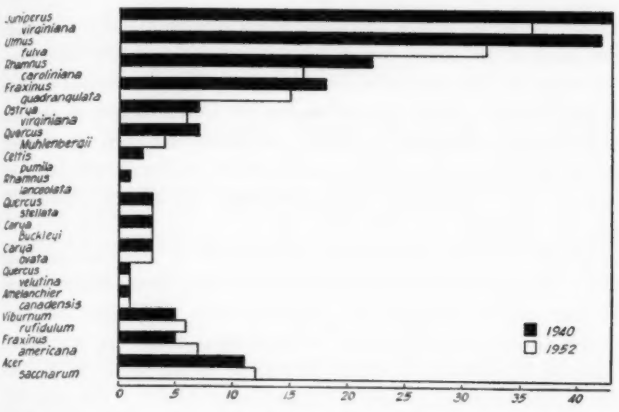

Fig. 8. Diagram representing relative numbers of plants of the species present on a quadrat in the Red Cedar-Chinquapin Oak Association for 1940 and 1952.

(Viburnum rufidulum). There were also seedlings of Blue Ash *(Fraxinus quadrangulata)*, White Ash *(F. americana)*, and small trees of Sugar Maple *(Acer saccharum)*.

The recent survey of the quadrat shows that about one-sixth of the Red Cedar trees were lost through competition and that the ones left had grown considerably. Some Chinquapin Oaks had also died but the remaining trees had made some growth. There was no change in the number of oaks and hickories and they also have grown. The single large Chittimwood has died. The greatest change is in the understory growth. Almost a fourth of the Slippery Elms have died and those left have scarcely grown either in diameter or height. Other understory trees as Indian Cherry and Hop-Hornbeam are less frequent but are growing vigorously. Lance-leaved Buckthorn and Dwarf Hackberry have died. There are a few more trees of Shadbush and Black Haw and they are thriving. The number of White Ash and Sugar Maple trees has increased slightly, but their seedlings and small trees are growing slowly. The Blue Ash, present mostly as small and seedling trees, has decreased in number, although the plants remaining are making moderate growth.

On this and the preceding quadrats, many specimens of Slippery Elm, White Ash, Red Cedar, Post Oak, Pignut Hickory, and Sugar Maple are only 4–5 feet in height. On casual inspection they give the appearance of young plants but actually they are 15–20 years old.

OBSERVATIONS ON FOREST SUCCESSION

Time-lapse studies presented in the foregoing forest quadrats and in the more general association maps in an earlier paper[3] have revealed significant facts concerning forest succession for the area under consideration. The conclusions reached for the local area may have a wider application for the Ozark region in general. One of the outstanding features brought out by this study has been the marked inability of most species to invade established associations except in the event of a catastrophe such as fire, lumbering, heavy pasturage, or abrupt changes in climate of considerable duration.

In the four quadrats described the invasion and decline of numerous seedlings have been observed. With almost no exceptions species have been able to invade established associations and to demonstrate vigor sufficient to suggest the possibility of their offering serious competition to established trees. It was found that the greater number of seedlings of species mentioned in the foregoing quadrat reports originated in the years following a major catastrophe, in this case the drought period of 1930–1936, which seriously weakened the trees in the region of the Arboretum Forest Preserve. During the time lapse of this study it has been observed that the existing associations continue in their "catastatic" state. Historical data indicate that a catastrophe will incite germination of seeds and start successful invasion of the disturbed association.

In any event, the association will be a happenstance entirely dependent upon the kind of seed immediately available and the peculiar requirements both for germination and survival of the seedlings. Even though the seedlings may survive and reach maturity they may not represent the best-adapted species for the site. However, no other species with similar requirements for germination were present at the time that the site was a frontier ready for invasion. Those plants surviving to seed-producing maturity will then become conspicuous in the forest association. It is believed that such species may often so completely occupy the site, filling shallow soils with roots and shading the soil surface with their tops, as to prohibit or retard seedling growth. The invasion of new plants in this established local association is thus prevented, and the association may be perpetuated for many generations and cover considerable areas. Plants unsuited for a particular site are often short-lived, as illustrated by the many forest trees used in landscape planting which mature early and become an easy victim of minor accidents. If the association is weakened, it will be vulnerable to seedling invasion. Better-adapted species may then enter if seed sources are adequate, or, lacking this condition, the growth of seedlings will comprise a regeneration of the existing association.

The Blue Ash *(Fraxinus quadrangulata)* has offered an excellent opportunity to study invasion as related to seed source. The early history of the area has shown that many Blue Ash trees had been cut for fire-wood and for farm-implement manufacture. When the Forest Preserve was established there were few trees of Blue Ash. Almost no seedlings were to be found in the Forest Preserve, but now many Blue Ash trees are fruiting abundantly, and the seedlings are invading adjacent open areas.

[3] Beilmann and Brenner, op. cit.

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